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Optimized Development, Characterization and Antimicrobial Evaluation of Bioactive Prodigiosin Potentials from Cephalosporin Resistant *Serratia marcescens*

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Abstract

Serratia marcescens is an opportunistic Gram negative nosocomial pathogenic bacteria. It accounts for 1%-2% of nosocomial infections confined to the respiratory tract, urinary tracts, surgical wounds in hospital settings and environments. This work was undertaken to isolate, characterize genotypically and phenotypically, *S. marcescens* for optimization prodigiosin production and evaluation of bioactive potentials. The test organisms were isolated from both clinical and non-clinical samples using streak and spread plate methods on peptone glycerol broth and agar. The isolate were characterized and evaluated for cephalosporin resistance using standard protocols. The pure prodigiosin was developed, optimized, extract, purify, characterized and dried using solvent-solvent extraction and other analytical methods. The prodigiosin metabolite was evaluated for phyto-constituents and antimicrobial potentials. The results showed an expressed red pigmented metabolite which appeared Gram negative bacteria and a dark, stained flagella on Scanning Electron Microscope (SEM). The biochemical tests and presence of corresponding gene from PCR *via* plasmid profiling confirmed the bacteria as *S. mercescens*. It was observed on the activity pattern, some level of resistance to first and second generation cephalosporins with average Inhibition Zone Diameter (IZD) of 5 mm but susceptible to third generation with maximum IZD of 25 mm. The optimized production yielded 0.2% bioactive principles and the metabolite subsequently, showed good antimicrobial activities with MIC and MBC between 8 and 4 mg/ml. Summarily, the activity pattern showed that the resistance could be as a result of plasmid DNA mediation and suggests the expression and production of some natural bioactive principle for diagnostics, management and treatment of some tropical diseases.

Keywords: *Serratia marcescens*; Cephalosporins; Podigiosin; Phyto-constituents; Antimicrobial activity

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Introduction

S. marcescens is an opportunistic nosocomial and facultative anaerobic Gram negative bacteria belonging to group of the *Enterobacteriaceae* [1], which was considered initially as non-pathogenic bacteria to human and other animals [2]. It was used as a biological marker because of expression and production of red pigmented secondary metabolite called prodigiosin. The red pigmented compound produced by this organism has also shown some important biological activities like anticancer on triple negative breast cancer (TNBC) [3,4], immunosuppression [5], antioxidant [6], antimicrobial [7]. But today, it is an established opportunistic pathogen that causes a wide range of infections as a result of gene DNA mediation and mutation. It accounts for 6.5% of nosocomial infections caused by Gram negative organisms in the intensive care unit, and ranks 5th among Gram negative

pathogenic bacteria [8]. The ability of *S. marcescens* is to cause disease is attributed to the production of virulent factors such as the pili [9], which enables it to colonize surfaces and cause infections. Some enteric bacteria have the capacity to hydrolyze the protein through fermentation and protect the host against diseases by producing secondary metabolites [10]. Therefore, this secondary metabolite produced by the proteolytic bacteria from Labeo rohita gut and was used in the fermentation of tannery fleshing to yield prodigiosin and its antagonistic activity against some invading pathogens and abnormal cells has been reported on the production of prodigiosin from tannery solid waste fleshing [11]. This *S. marcescens*is as an opportunistic infectious agent can be implicated majorly among immunocompromised patients and drug addicts and other predisposing factors [12]. Recently, nosocomial infections caused by this organism are most times difficult to treat as a result of some intrinsic resistance factors of this species harbored in plasmid DNA as the marker to acquire further resistance to many groups of antimicrobial agents such as cephalosporins, β-lactams, aminoglycosides and fluoroquinolones [13]. *S. marcescens* and other *enterobacteriaceae* have exhibited common ways of becoming resistant to some classes of antibiotics by acquiring gene mutation as a common derive with exception of *S. marcescens* which developes unique production of reddish prodigiosin metabolite [14], and other enzymes like β-lactamases, AmpC-type cephalosporinase, and carbapenemases which interfere with the permeability of the outer membrane leading to modification of the target site for the penicillin binding proteins, efflux mechanism and structural alterations of the GyrA protein [15].

Materials and Methods

Microscope, Autoclaves (Kumar sales corp, Mumbai, India), Incubators (Heraeus and Co), Oven dryer (Gallenkamp and Co), Refrigerators, Weighing balance (Measure Tech), Wire loop, Bunsen burner, Droppers and Glasswares. The chemicals and reagents were Lugol Iodine (Merck, Germany), Safranin (Merck, Germany), Alcohol, Gentian violet, (Merck, Germany), Sterile water, Kovac's reagent, Glucose, Lactose, Simon's citrate, Phenol red indicator, Peptone water (Oxoid, England), Nutrient broth (Oxoid, England), Agar agar, Müeller Hinton's agar (Lifesave Biotech, USA), Antibiotic disc [cefuroxime, ceftazidime, ciprofloxacin (Oxoid, England), Azithromycin, Ceftriaxone].

Cultivation, isolation, purification of *S. marcescens*

S. marcescens was isolated from urine samples by adopting agar surface streak method [16] through nosocomial infection using Serratia differential medium (Himedia) and peptone glycerol agar. The urine samples were serially diluted in a sterile normal saline and the diluted clinical sample streaked across the surface of the over-dried sterile agar plate using sterile wire loop and incubated at 27oC in the absence of light for 24 hours. Similarly, the cultivated isolates were further isolated and purified by transferring a single colony of the pure test organism unto a fresh medium for further streaking and incubation as above to obtain pure isolates of the *S. marcescens*. The pure isolates were characterized phenotypically through colony morphology, microscopy and molecular characterization.

Colonial evaluation and characterization of *S. marcescens*

Bacteria colonies grown on the Peptone Glycerol Agar (PGA) media are visible mass of microorganisms originating from a single mother cell, and constitute a clone of bacteria cells that are genetically alike and were characterized for Colony morphologies for identification through size, shape, texture, opacity, elevation, pigmentation and effect on growth medium.

Microscopic examination and identification of *S. marcescens*

The simple and Gram stains were adopted for fast and convenient ways to determine cell shape, size, arrangements and Gram character of bacteria using 100X objective lens of compound light microscope with oil immersion [17]. All were applied in the identification of the structural appearance of the organism.

Scanning Electron Microscopy (SEM)

The internal structure of *S. marcescens* was properly examined under scanning electron microscope for clear structural identification.

Biochemical tests

Biochemical tests like indole, citrate, gelatin hydrolysis, nitrate reduction, oxidase, pigments production as well carbohydrates fermentation like lactose, fructose, sucrose, sorbitol, glucose, galactose, tartrate were employed in confirmation of the test organism.

Enzyme hydrolysis test

Various enzymatic hydrolysis tests such as gelatinase test, casein hydrolysis test, lipid hydrolysis test and arginine dehydrolase were carried out.

Molecular Characterization of *S. marcescens*

Polymerase chain reaction confirmation

The polymerase chain reaction was carried out by following an established method [18]. DNA sample of strain of *S. marcescens* primer 16s RNA forward primer 5'TAG GGA AGA TAA TGA CGG 3', Reverse primer 5'CCT CTA TCC TCT TTC CAA 3' 10X amplification buffer contains 500 mm KCl, 100 Mm HCl (pH 8.3), 15 Mm $MgCl_2$, DNTP solution (20 Mm) having pH 8.0 Taq DNA polymerase, thermal cycler programmed with desired amplification protocol, ethidium bromide 0.5 mg/ml, 1500 bp DNA marker. The 50 ml reaction mixture will be transferred into a 0.5 ml microfuge amplification buffer 5 ml, 20 Mm Solution of DNTP (pH 8.0) 1 ml, 20 Mm forward primer 2 ml, nuclease free water 33 ml, DNA sample 5 ml, total volume 50 ml. The reaction mixture was gently centrifuged at 4000 rpm for 5 minutes in order to settle down the mixture which have got layered on the walls of the microfuge amplification tube. The reaction mixture was then be placed in the cycler fitted with a heated lid, the nucleic acids was amplified

by setting denaturation at 94 degrees/1 min, annealing at 55 degrees for 30 seconds and extension at 72 degrees for 1 minute in the thermal cycler and the number of cycles is 30 cycles. After polymerase chain reaction, 15 ml of amplified DNA products was run in an agarose gel electrophoresis by preparing 1.5% agarose gel and the DNA bands viewed under the transilluminator and the distance and sizes, recorded.

Plasmids Isolation and profiling

Plasmids isolation was carried out based on the rapid alkaline extraction procedures for screening of recombinant plasmid DNA [19,20]. Agarose gel electrophoresis was used to resolve the extracted plasmids: standard DNA molecular weight marker (0.12-23.1 kbp) of bacteriophage lambd hind 11 (Roche diagnostic GmbH) will be used as standard DNA marker. Plasmid composition of the semi purified clear lysates was determined by horizontal agarose gel electrophoresis [21]. This included the use of 0.8% agarose slab gels in tris borate EDTA buffer. The gel was stained with 14 µl ethidium bromide for 45 minutes and then photographed under UV light transillumination before comparing it with the standard marker [22].

Antibiotic sensitivity and resistance evaluations

The strains were tested for antibiotic activity pattern, thus: the antibiotics sensitivity/resistance profiles (antibiogram) of the isolates were determined by the disk diffusion method. The Inhibition Zone Diameters (IZDs) produced on the inoculated Mueller-Hinton agar plates by the antibiotics disc were measured and recorded. The activities of ofloxacin, ceftriazone, amoxicillin, gentamicin, imipenem and co-trimoxazole were compared with their respective break points.

Optimization and production of prodigiosin from *S. marcescens*

The purified culture of *S. marcescens* was grown en masse on a sterile peptone glycerol agar plate using surface spread plate method [16]. This was repeated on several numbers of 30 cm Petri-dishes respectively and incubated in dark condition at 30°C for 48 hours.

Extraction and concentration of prodigiosin from *S. marcescens*

The surface culture scraping method of prodigiosin extraction was adopted [23], the mass culture of the *S. marcescens* on the PGA media was scooped into 100 ml of absolute ethanol, agitated vigorously to extract the red pigment into the ethanol as a soluble solvent [24]. The liquid solution of *S. marcescens* ethanol was filtered using Whatman filter paper (number 1) to remove dense culture bacteria and agar debris from the solution. The solution was then industrial centrifuged at 5000 revolutions per minute (rpm) for 10 min for further separation and clearer solution. The red pigmented supernatant was subjected to rotary evaporation (BUCHI 011, Laboratorums® with Water Bath CH-9230, Switzerland) as shown in Figure 1 to remove the excess alcoholic solvent leaving the crude concentrated extract of prodigiosin behind for further purification and lyophilizaton.

Figure 1: The concentration by rotary evaporation.

Purification and spectrophotometric analysis of prodigiosin extract

The concentrate was then purified using column chromatography as shown in Figure 2. The silica gel of 2000 w mesh (BDH Chemicals, England) was used as stationary phase, while the mobile phase was a mixture of ethyl acetate, chloroform and methanol in the ratio of 2:1:1, respectively. The prodigiosin extract solution was added at the top of the gel in the column and fractions were eluted by adding the mobile phase and then collected in fractions for several minutes. The prodigiosin extract was evaluated for its purity using UV-Vis spectrophotometer (Schimadzu Corp, Japan) at a wavelength of 535 nm. The pure fractions at the wavelength of 535 nm were pooled together and subsequently freeze dried in a bulk freeze dryer (Biopharma, Winchester, SO23 0LD, UK). The prodigiosin sample was then stored in an amber colored container to maintain its efficacy, which can be affected by ultra-violet radiation in a refrigerator [25].

Figure 2: Colum chromatographic purification of the prodigiosin solution, this process enhanced the removal of the excess extracting solvent and separated the pure PG from other contamination agents.

Phytochemical evaluations of the prodigiosin compound

The powdered aqueous extract was subjected to phytochemical tests using established and standard procedures for the determination of alkaloids, tannins, saponins, glycosides, flavonoids, carbohydrates, fats and oil [26].

In vitro bacteriostatic concentration (MIC) and bactericidal concentration (MBC) evaluations of prodigiosin.

Sensitivity and Minimum Inhibitory Concentration (MIC)

The *in vitro* sensitivity evaluation of the extracted pure prodigiosin against different bacterial and fungal isolates was done using agar well diffusion [27], while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using agar dilution and micro broth dilution methods [28].

Results

The colonial, microscopic and biochemical characterization of the test organism

Out of many samples isolated clinically from catheter urine samples and environmentally (ant-hill) collected from Diogbe town in Igbo-Etiti Local Government Area, Enugu State and Bishop Shannahan hospital, Nsukka respectively, eight samples were chosen for colony morphological studies based on the presence of growth and their distinct distributions. These samples were coded as R_1, R_2, R_3, MB_{24}, MB_{26}, MB_{29}, MB_{30}, MB_{31} for easy identifications in the process of characterization and possible confirmation. The colony morphological characteristics of the isolates in Figures 3a and 3b were studied with several observations and recorded in Table 1. R_2, R_3, MB_{26} and MB_{30} chromogenically produced red pigments which showed characteristic color similarity with *S. marcescens* peptone glycerol media after maintaining all the necessary conditions for its growth.

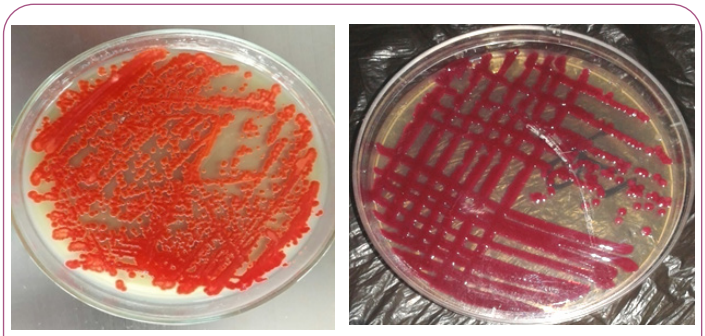

Figure 3: The colony morphology (a) the clinical; (b) environmental isolates, this revealed the unique colony characteristics of S. marcescens which applies in phenotypic identification and characterization of bacteria cells.

The morphological characteristics as recorded in the Table 2. phenotypically showed the structure and Gram character of the Serratia spp as reported [29]. The suspected *S. marcescens* isolates, when subjected to gram staining and microscopic examination on the basis of their Gram character, shape and arrangements were shown to be Gram negative organisms without spore formation and also rod-like in shape with clustered arrangements as shown in Figure 4a. The extended review under the Scanning Electron Microscope (SEM) in Figure 3b, showed clear internal distinct structure of the *S. marcescens*.

Codes	Gram reaction	Shape	Arrangement	Inference
R2	Negative	Short rod	Clusters	Gram negative rod
R¬3	Negative	Short rod	Clusters	Gram negative rod
MB26	Negative	Short rod	Clusters	Gram negative rod
MB29	Negative	Short rod	Clusters	Gram negative rod
MB30	Negative	Short rod	Clusters	Gram negative rod

Table 1: Colonial morphological characterization of the test organism.

Sample source	Colony characteristics	32	32	32	32	32	32
	Entire	Chromogenesis	Opacity	Elevation	Surface	Consistency	Odour
Clinical (Urine-R)	Circular	Reddish pigment	Opaque	Raised	Glittery	Butyrous	Present
Environmental (Ant-hill-MB)	Circular	Dark Reddish pigment	Opaque	Raised	Glittery	Butyrous	Present

Table 1: Colonial morphological characterization of the test organism.

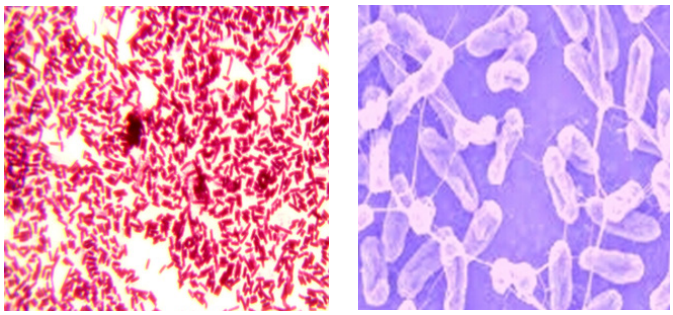

Figure 4: The microscopic characterization of S. marcescens; (a) Gram reaction of S. marcescens, (b) Scanning Electron Microscope (SEM) structure of the isolates. This technique X-rayed the structural morphologies of the Serratia bacteria used.

The Biochemical and sugar utilization tests were conducted to determine the biochemical and enzymatic responses of the isolated test organisms through their metabolic products of fermentation in the sugars; lactose, glucose and citrate utilization, and indole reaction. In Table 3, it also showed that the selected test organisms responded positively to the sugar fermentation and utilization studies as in the biochemical test results. R_2, R_3, MB_{26}, MB_{29} and MB_{30} showed a negative result to indole test which could suggest that the enzyme tryptophanase may not be expressed by the organism to convert the amino acid, tryptophan to indole as a red ring on the bacteria culture with indole reagent (Kovac's) [30].

Biochemical and sugar utilization tests

The S. marcescen plasmid DNA profile and cephalosporin activity patter: The aggregation of larger sized genes as shown in Table 4, with up to 18.31 kb, as a complex nature of its biosynthetic pathway, can render the expression of some genes in the organism for the prodigiosin production with different sizes when compare with the linear DNA standards [31]. Figure 4a showed presence of distinct plasmid DNA bands with different molecular weights and mobility rates as shown in Figure 4b, for the standard plots in comparison with the standard DNA ladder against molecular weight of standard which is used to determine the molecular weight of standard with known mobility weight in millimeter.

Codes	Sugar utilization			Indole test
	Lactose	Glucose	Citrate	
R2	Positive	Positive	Positive	Negative
R3	Positive	Positive	Positive	Negative
MB26	Positive	Positive	Positive	Negative
MB29	Positive	Positive	Positive	Negative
MB30	Positive	Positive	Positive	Negative

Table 3: Biochemical tests.

Plasmid DNA standard (ladder)		Plasmid DNA profile of test organisms		
Mobility weight (mm)	Molecular weight (Kb)	Isolate codes	DNA mobility weight (mm)	DNA molecular weight (Kb)
0.2	20	S1 (R2)	0.45	14.5
0.9	15	S2(MB26)	0.375	16.4
1	8	S3(R¬3)	0.4	15.81
1.1	6	S4(MB29)	0.35	17
1.2	5	S5(MB30)	0.35	18.31
1.3	4			
1.4	3			
1.5	2			
1.8	1.5			
2	1			
2.4	0.5			

Table 4: Plasmid DNA profile of ladder (standard) and test isolates.

Cephalosporin resistant activity patter: From the IZDs obtained from activity pattern in Table 5, also all the test organisms, except MB_{31} were more susceptible to ciprofloxacin than all the other antibiotics. However, MB_{29} had the highest susceptibility to ciprofloxacin with a mean IZD of 28 ± 21 mm. The susceptibility of test organism R_1 could be said to be in this range CIP>SYN>AZI>CXM>CTZ which implies that the diseases caused by this organism can better be managed with ciprofloxacin and ceftriaxone respectively. The susceptibility of R_2 can be summarized to be in this range CIP>AZI>SYN=CXM>CTZ. It was observed from the result that R_2 is cephalosporin resistant, only susceptible to ciprofloxacin and azithromycin but resistant to both second and third generation cephalosporins used in this study. The susceptibility of R_3 could be summarized thus CIP>SYN>AZI>CTZ>CXM. R_3 being the reference organism, showed a good response to ciprofloxacin, ceftriaxone, ceftazidime and azithromycin. The activity pattern against MB_{24} is summarized thus: CIP>SYN>AZI>CTZ>CXM while it maintained resistance to ceftazidime and cefuroxime as cephalosporin generations. The susceptibility pattern of MB_{26} is also summarized as CIP>SYN>CXM>CTZ>AZI while that of MB_{29} was CIP>SYN>AZI>CXM>CTZ being resistant to cefuroxime and ceftazidime but susceptible to ceftriaxone and ciprofloxacin; it was sensitive to azithromycin. MB_{30} and MB_{31} had sensitivies as follows: CIP>SYN>AZI>CXM>CTZ and SYN>CTZ>CXM>CIP=AZI respectively with MB_{31} being susceptible to cephalosporins unlike the other isolates.

Sample codes	SYN	CXM	CTZ	CIP	AZI
	Inhibition zone diameters (mm)				
R1	17 ± 0.9	14 ± 11	25 ± 15	15 ± 11	15 ± 0.9
R2	10 ± 0.7	0 ± 0.00	8 ± 0.74	25 ± 0.6	20 ± 10
R3	8 ± 0.8	5 ± 0.92	22 ± 11	23 ± 0.9	17 ± 11
MB24	19 ± 0.7	5 ± 11	6 ± 0.9	27 ± 20	9 ± 11
MB26	10 ± 1.0	26 ± 21	18 ± 13	27 ± 21	15 ± 14
MB29	12 ± 0.8	6 ± 0.87	5 ± 0.84	28 ± 21	17 ± 14
MB30	20 ± 0.4	9 ± 13	5 ± 0.81	25 ± 13	12 ± 13
MB31	15 ± 1.1	20 ± 16	25 ± 12	0 ± 0.00	0 ± 0.00
KEYS SYN= Ceftriaxone (30 µg), CXM=Cefuroxime (30 µg), CTZ=Ceftazidime (30 µg) CIP=Ciprofloxacin (5 µg), AZI=Azithromycin (5 µg)					

Table 5: Activity pattern (Antibiogram) of isolates against *Serratia marcescens*.

Optimized production, extraction and characterizations of prodigiosin from *S. marcescens*: The isolated, purified and characterized *S. marcescens* as shown in Figure 5a expressed and produced red pigmented metabolites on the peptone glycerol agar (with composition of 5% peptone, 1% glycerol and 15 g/l agar at pH 7) with increased dense coloration after extended 48 h of incubation under dark condition and a reduced temperature. The alcoholic extract of the prodigiosin showed a clear red pigmented solution from three different ratio combinations of methanol, ethylacetate and chloroform (2:1:1). The extracting solvents recovered of about 90% of their original volume at the respective boiling points from prodigiosin solution through the reflux condensation mechanism. Figure 5b showed fractionated, purified and dried prodigiosin with 0.2% yield. The standard 10 mg/ml concentration of PG with single peak at 535 nm under spectrophotometric analysis. The dried stock sample was preserved in dark amber colored container due its light sensitivity in a refrigerator.

Phytochemical constituents of the prodigiosin metabolites: The results of phytochemical and antimicrobial analyses in Table 6 showed presence of some secondary metabolites such as starch, resins, protein, flavonoid and fat.

***In vitro* bacteriostatic and bactericidal activity of prodigiosin:** The antimicrobial activity pattern of the prodigiosin in comparison with standard ciprofloxacin (CIPR) 2.0 µg against some selected bacteria isolates in Figures 6a, b , and c showed that the metabolite had activity against both Gram positive and Gram negative bacteria. The inactiveness of Prodigiosin against *Pseudomonas* aeruginosa with the minimal activity against *E. coli* other gram negative bacteria, confirmed some report where the metabolite have proven insignificant or no activity on such group of organisms.

The MIC and MBC of prodigiosin with standard antibiotic: This metabolite has shown to be active against gram positive bacteria with more effect against Bacillus species and Staph. aureus as shown in Figures 7 and 8 of the MIC and MBC respectively. This bioactive metabolite named prodigiosin has been describe as an intracellular antibacterial red pigment [32], relating to its activities against bacteria cells.

Phytochemical constituents										
Test	Tannin	Starch	Saponin	Resin	Reducing sugar	Proteins	Flavonoids	Glycosides	Alkaloids	Oil
Results	--	+++	--	++	--	+++	+++	+	++	+++

Table 6: The Phytochemical components of extracted yam samples.

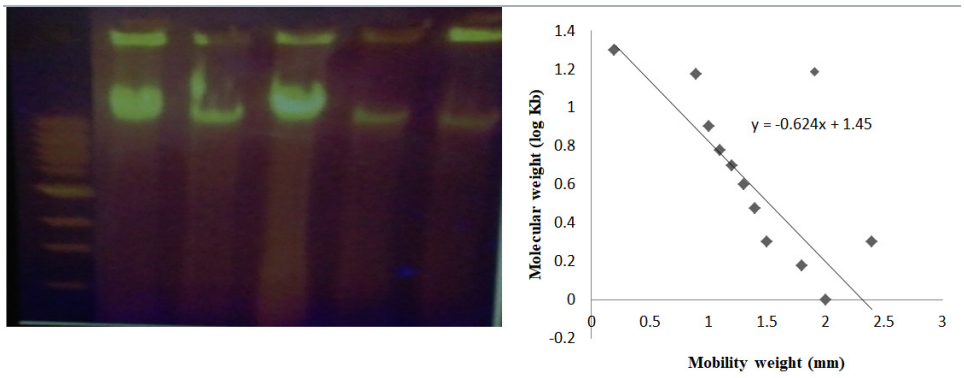

Figure 5: The plasmid DNA isolates and standard profile plot; (a) the different lanes as S1-5 codes for the S. marcescens: 2 plasmids=13.70 kb, Lane S2 (MB26)-S. marcescens: 2 plasmids=14.50 kb, Lane S3 (R¬3)-S. marcescens: 2 plasmids=16.40 kb, Lane S4 (MB29)-S. marcescens: 2 plasmids=15.81 kb, Lane S5 (MB30)-S. marcescens: 2 plasmids=17.00 kb, (b) Standard plasmid DNA plot as maker.

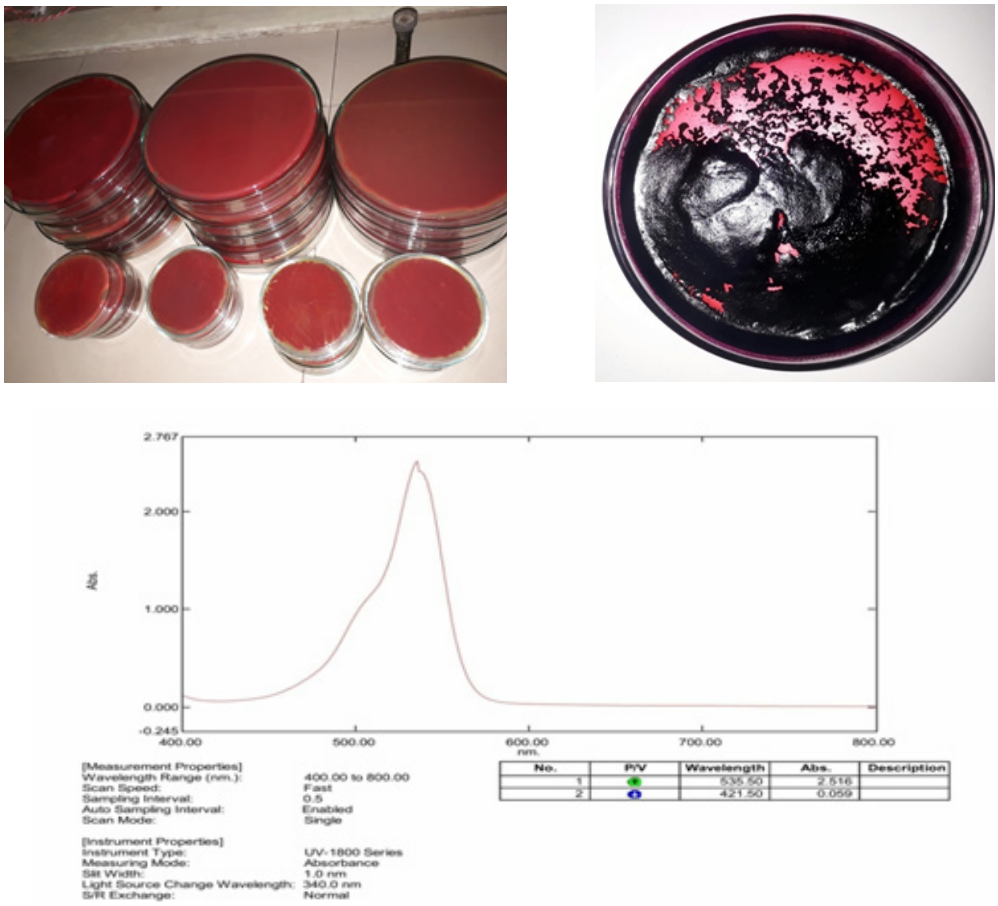

Figure 6: The development and optimized prodigiosin productio; (a) Optimization of the expressed bacteria prodigiosi production on Peptone Glycerol Agar (PGA) medium, (b) Extract and dried prodigiosin compound, (c) UV-visible spectra analysis of the prodigiosin isolate.

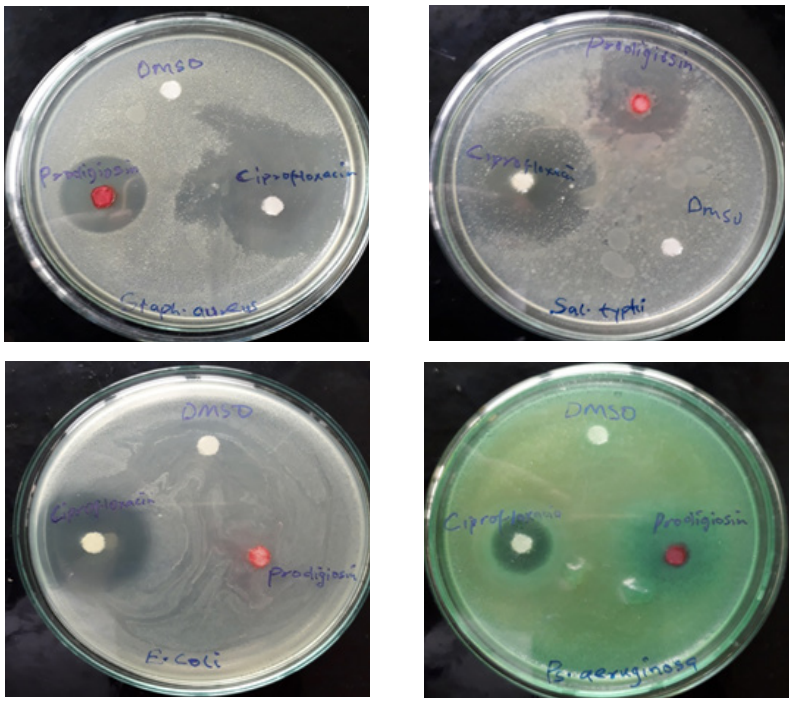

Figure 7: The Antimicrobial activity pattern of prodigiosin against some bacteria; (a) Staphylococcus aureus, (b) Salmonella typhi, (c) Escherichia coli and (d) Pseudomonas aeruginosa.

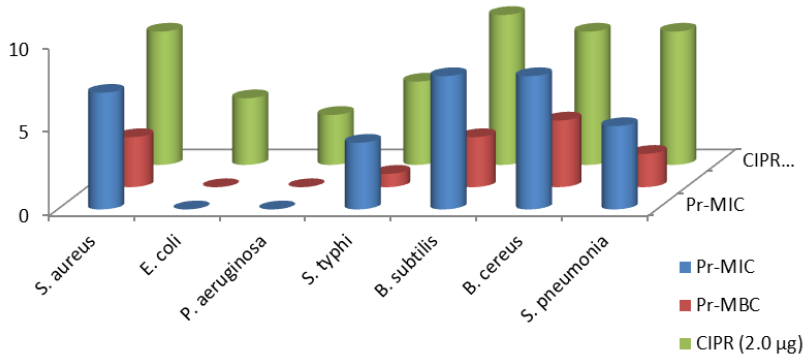

Figure 8: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of prodigiosin against the tested bacteria cells.

Discussion

The red pigment produced by the organism suggests the presence of prodigiosin expression which is a secondary metabolite associated with some Gram negative bacteria, especially pathogenic once [24]. The colonies showed on the Peptone Glycerol Agar (PGA), a glittery and shiny surfaced, round colonies with entire margin [33]. The others produced no red pigment but showed similar morphological resemblance to *S. marcescens*. It is worthy of note that *S. marcescens* under some conditions may not produce the red pigment [34], which can be as a result mutation or condition of growth. Other morphological characteristics that were studied on the plate included: Size, opacity, elevation, consistency and odor. This gram negative organisms have been shown to possess intrinsic inability to retain the primary stain but complexes with certain compounds and dyes due to their cell complex of peptidoglycan [35]. The internal structure of *S. marcescens* was properly reviewed under scanning electron microscope for subsequent identification as a Gram negative motile bacterial isolates due to the presence of flagella as its ability to colonize both its human and environmental host [36]. The biochemical responses of the suggests that the bacteria produced lactase and phosphatase enzymes which broke down the sugar bond and thus released free glucose which can be easily utilized by the organism to generate its energy and acid which changes the pH of the medium leading to color changes and this process is best described by the degradation of glucose by Embden-Meyerhof pathway [37]. Some strains of *S. marcescens* have been reported non fermenters of some sugars like arabinose in peptone water but can oxidize for carbon starvation source of energy as one of the reasons of its involvement in destruction of food substances made of sugars and starch [38]. Similarly, *S. marcescens* showed negative response to indole test because it lacks the ability to produce tryptophanase which is the enzyme that converts tryptophan to indole pyruvate and then to indole [39]. Consequently, the gene chemical response of this organism which usually harbor resistant factor in bacteria and other organisms is plasmid DNA which in most cases are difficult to establish the size limits of plasmids or the actual size distribution in any organisms as a result of isolation protocols which has shown to be better with the smaller plasmids than the larger ones [40]. Bacterial prodigiosin production has recently withnessd from the human nosocomial and opportunistic pathogens such as *S. marcescens* has reported [41], due to the encoded gene expression [42]. Therefore, the presence of the larger sized plasmid DNA could be responsible for resistance to antibiotics. According to NCCLS break point of the selected cephalosporins on *S. marcescens*, R_2 and R_3 were considered to be resistant to cefuroxime and other member drugs with mean IZDs of 9 mm and 5 mm respectively. It can be noted that the resistance to first and second generation cephalosporins by *S. marcescens* could be as a result of presence of resistant plasmid DNA gene found in the organism with extended-spectrum β-lactamase enzymes capable of resisting other antibiotics [43]. Therefore, the nosocomial infections caused by this organism are often shown hard to treat due to the presence of intrinsic plasmid DNA resistance factor of this species and its mutational changes to acquire further resistance to many groups of antimicrobial agents [44]. This presence of the red pigment suggested the expression and production of prodigiosin [45]. This prodigiosin is expressed as tripyrrolic red-colored secondary metabolites of prodiginines from some of the Gram negative bacteria with some level of bioactive principles [46]. Prodigiosin as a natural biosynthentic metabolite, is naturally synthesized from amino acid and acetate building blocks [47,48] by different bacterial strains including species of Serratia [49]. The prodigiosin concentrate showed red dry sticky sample after 48 h inside dark oven dryer at 38°C, the texture of the metabolite suggesting the presence of fat, oils and other secondary metabolites which are responsible for most of the biological activities [11]. The medicinal value and biological activities of the prodigiosin as a natural product are perhaps due to the presence of these various secondary metabolites such as; alkaloids, flavonoids, glycosides, saponins, sterols and phenols [50]. Saponins as a natural analgelsic, posses anti-inflammatory, antioxidant properties which increases immune system with haemolity effect [51,52]. The presence of alkaloids could confirm that prodigiosin possess an antimicrobial properties which could be potential remedy for some infective diseases of bacteria. Glycosides are biological active agents like hormones, sweeteners, alkaloids, flavonoids, antibiotics which improves pharmacokinetic parameters and activities through glycosidic residues [53]. Similarly, the antibacterial activity of prodigiosin

metabolite has been reported for its ability to permeate through the outer membrane of the organism whereby inhibiting the target enzymes like topoisomerase IV and DNA gyrase leading to inhibition of the cell growth [54]. The important finding in this study was the high prevalence of DNA, which was plasmid mediated as seen among some various strains of *S. marcescens*.

Conclusion

It can be concluded that the clustered gene could be responsible for the antibiotic resistance observed with some strains of the organism against some tested antibiotics. The production of prodigiosin was optimized by chosen appropriate growth medium and observation of the required conditions and measures for the growth and expression of *S. marcescens*. The expressed bioactive metabolite by *S. marcescens* in some reports have shown potent biological activities that can help in tackling most of the ravaging diseases. It is an effective proapoptotic agent against some breast cancer cell lines like triple negative breast cancer, with multiple cellular targets of multi-drug resistant cells with little or no toxicity, antioxidant, antimalarial and other microbial killer agents. Some of the phytoconstituents present in the metabolite has been widely used in food production industries as colour additives for sensorial values, in cosmetic industries for their sun screening effect and drug production industries as colour additives and indeed can be used as an active pharmaceutical ingredient.

Author's Contribution

THG and IM: Conceptualized and design of methodology; Conducted experiments and investigation process. GDB and SBP contributed new reagents or analytical tools. AAA, ECI and GDB Redesigned, supervised the research work. THG and AAA analyzed data. THG and ECI and GDB wrote the manuscript. All authors read and approved the manuscript. We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

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Ethical Statement

This research does not involve human or animal object, therefore it does not require ethical approval or clearance during the course of the experiments.

Statement of Conflicts of Interest

All the authors have declared that, there is no conflict of interest in the course of the research.

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