



Studies on Production and *In Vitro* Antibacterial Potentials of Violacein from *Chromobacterium Violaceum* Isolated from Otamiri River in Owerri, Imo State

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Abstract

Chromobacterium violaceum was isolated from Otammiri River in Owerri, Imo State. The organism was isolated using nutrient agar and identified based on cultural and biochemical characteristics. The isolate produced violacein pigment on nutrient broth and the violacein produced was quantified and extracted with ethanol. The effects of different temperatures (4°C, 25°C, 30°C, 37°C, 40°C, 52°C), pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0), incubation period (12hr, 24hr, 36hr, 48hr, 60hr, 72hr, 84hr, 96hr), carbon sources (dextrose, fructose, glucose, maltose, lactose, sucrose, starch), nitrogen sources (ammonium nitrate, ammonium chloride, yeast extract, beef extract, peptone) on violacein production by *C. violaceum* isolated were examined. Antibacterial activity of gram negative and gram positive organisms was done and the MIC of violacein was determined. The amount of violacein produced by *C. violaceum* was 34.98mg/l. The production of violacein was increased with increase in temperature reaching its maximum value at 37°C. The optimum pH and incubation time for the production of violacein from the isolate were pH 6.5 and 72hr. Glucose and yeast extracts were most efficient carbon and nitrogen sources for the violacein production from *C. violaceum* isolated. The violacein was highly sensitive to *S. aureus* and *Escherichia coli* with inhibition zone of 15mm resistant to *Klebsiella aerogenes* and *Pseudomonas aeruginosa* with no inhibition zone. This study revealed that violacein has antibacterial activity against Gram positive and Gram negative organisms and provides knowledge of factors influencing the production of violacein. It also revealed the MIC of violacein and the MBC against different bacteria. The findings from this work could be of interest and suggest the need for further investigations in terms of toxicological studies and purification of active components with a view to using violacein in novel drug development.

Keywords: Violacein; Antibacterial Potentials; River; Water; *Chromobacterium violaceum*; Otammiri

Abbreviations: PBS: Phosphate Buffer Saline; NB: Nutrient Broth; BHI: Brain Heart Infusion Broth; PB: Peptone Broth; KIA: Kliger Iron Agar; MIC: Minimum Inhibitory Concentration

Introduction

Chromobacterium violaceum is a saprophyte found in soil and water habitat of tropical and subtropical areas of several

continents, normally considered non-pathogenic to humans but they can be opportunistic pathogen with great virulence. *C. violaceum* is a species of bacteria that are large, motile, gram - negative, beta - proteobacterium, non - spore formers. They are 0.6 - 0.9mm x 1.5 - 3.0mm in size and exhibit bipolar staining. It is positive for catalase and oxidase reaction. Motility of *C. violaceum* is achieved by means of a single polar flagellum and up to four antigenically and structurally distinct lateral flagellae. They are facultative anaerobes with

agrowth temperature range of 15°C – 40°C. Optimal growth is achieved at 30°C – 35°C, characteristically they produce violet colonies on nutrient agar and usually on MacConkey agar. The violet colour comes from pigment violacein. Colonies are low, convex, violet, smooth and not. A non - diffusible pigment is produced by this Gram – negative organism, called violacein [1]. This violacein has various antagonistic properties and it is bactericide [2], a trypanocide [3], and tumoricides [4] and in addition, it has anti-viral activities [5]. Violacein is a purple pigment produced by free environmental bacterial species, especially by *Chromobacterium violaceum*. This compound has several biological activities, including antitumoral and apoptosis – inducing properties in cancer cells, antioxidant, leishmanicidal, trypanocidal, antifungal, weak antiviral [6] and antimalarial effect [7]. As an antibacterial agent, violacein showed activity against *Mycobacterium tuberculosis* [8].

One of the main issues regarding the therapeutic uses of violacein is its toxicity *in vivo*, as this compound is cytotoxic to several tumour models, such as leukemic cells, colorectal tumors, human intestinal epithelial cells and ascites tumor models [6]. Very recently, Bromberg, et al. [9] have shown that violacein causes oxidative stress and cell death by apoptosis in Ehrlich ascites tumor cells. Violacein has strong antibacterial effects making it a promising candidate as an antibiotic. Moreover, when administered in combination with other antibiotics, the impact is more effective in fighting bacteria than the use of antibiotics alone [10]. This is of particular interest in light of recent antibiotic – resistant strains of pathogenic bacteria, such as MRSA. Also, the antiprotozoan properties of violacein could be exploited to treat disease in humans, such as in malaria and leishmaniasis [11]. The most studied clinical use of violacein, however, is its being a potential cancer therapeutic. Violacein has been tested against various cancer cell lines where it has shown cytotoxicity at IC₅₀ values that mostly range in the submicromolar concentrations. The effects of violacein were also shown to be specific for the cancer cell line tested as two colorectal cancer cell lines, Caco – 2 and HT – 29, were differentially susceptible to violacein [12]. Since violacein is cytotoxic towards non cancer cells as well [13]. The critical factor for its clinical use against cancer is that it is more toxic to cancer cells than to normal cells. One study demonstrated that violacein induced apoptosis in HL60 cells (IC₅₀ = 700nM), a cancer cell line used as a model to study myeloid leukemia. However, normal lymphocytes were unaffected at the concentrations tested, further asserting violacein's use as a putative cancer therapeutic [14]. Even though violacein is a promising agent as an antibiotic and a treatment for cancer, the biological mechanisms behind these actions remain elusive. Before we can stamp violacein as a natural therapeutic for numerous kinds of disease, it is imperative to understand its mechanisms of action at the cellular and

molecular levels. Conclusions on violacein's mechanism of action are clearly scanty at this point [14]. The issue of drug resistance has become a serious challenge in the present day medical practice, effort is now geared towards the production of new and potent antibacterial. This research intends to evaluate the optimization of violacein production and its antibacterial potentials. Thus this will help in the development of a new drug for the treatment of infections. This makes this study very necessary and important.

Materials and Methods

Study Area

The Otammiri River is one of the main rivers in Imo State, Nigeria. The river takes its name from *Otammiri*, a deity which owns all the waters that are called by its name, and which is often the dominating god of Mbari houses. The river runs south from Egbu past Owerri and through Nekede, Ihiagwa, Eziobodo, Olokwu Umuisi, Mgbirichi and Umuagwo to Ozuzu in Etche, in the Rivers State, from where it flows to the Atlantic Ocean. The length of the river from its source to its confluence at Emeabiam with the Uramiriukwa River is 30 kilometres. The Otamiri watershed covers about 10,000 square kilometres with annual rainfall of 2,250 to 2,500 millimetres (89 to 98 in). The watershed is mostly covered by depleted rain forest vegetation, with mean temperatures of 27°C (81°F) throughout the year. Conversion of the tropical rainforest to grassland with slashes and burn practices is degrading soil quality. The Otamiri is joined by the Nworie River at Nekede in Owerri, a river about 9.2 kilometres (5.7 mi) long. The Nworie River is subject to intensive human and industrial activities, and is used as a source of drinking water by the poor populace when the public water system fails. The Nworie is polluted by organic wastes but in 2008 was not above acceptable levels of chemical pollution. Waste management in Owerri is inefficient and contributes to pollution of the river. Most of the wastes from Owerri are dumped at the Avu land fill in Owerri West on the Port Harcourt highway, which creates a high concentration of phosphate and nitrate in the Otamiri. South of Owerri the river flows through an alternating sequence of sands, sandstones and clay-shales. Random sand samples from the bank of Otamiri River between Chokocho and Umuanyaga, Etche Local Government Area, Rivers State showed that 86 percent of the sand particles are within the ideal range for glass making.

Sample Collection

200ml of water sample was precautiously collected from Otamiri River in Owerri into a sterile universal container. The samples were sent immediately to the laboratory for analysis.

Laboratory Analysis of Test Organisms

Isolation and Identification of *Chromobacterium violaceum*: Ten milliliter of water sample was transferred into a sterile centrifuge tube and centrifuged at 60,000nm for 10mins. After centrifugation, the supernatant was discarded and the deposit was resuspended in 3ml nutrient broth. The broth was then incubated at 37°C for 24hrs [15]. After incubation, the broth culture was serially diluted to 10⁻³ with sterile phosphate buffer saline (PBS). Diluted samples were then cultured on nutrient agar plates and incubated at 37°C for 48hrs [16]. The plates were examined for microbial colonies with violet pigmentation. Typical colonies were purified by successive streaking on nutrient agar. The isolates were maintained on nutrient agar slants, stored at 4°C and subcultured at 14 day intervals.

Phenotypic Characterization: Isolate was identified based on its cultural and cellular morphology after which it was subjected to various physiological and biochemical tests such as Gram stain, catalase test, oxidase test, indole test, motility test, citrate utilization, urease test, gelatin liquefaction, Vogues Prouskauer test, Methyl red test, growth on KIA, carbohydrate fermentation test (glucose, lactose, sucrose, galactose, maltose, arabinose).

Extraction and Quantification of the Pigment Violacein: The organism was cultured into 200ml nutrient broth and incubated at 37°C for 48hrs. After the incubation, the broth was taken for the pigment extraction using, Vishnu and Palaniswamy [17] method. Ten milliliter of fermented broth was collected into a sterile centrifuge tube, centrifuged at 10,000rpm for 5mins and the supernatant was discarded. The cell deposit was then rinsed with deionized water followed by centrifugation at 10,000rpm for 5mins and decanted the supernatant. Five milliliter ethanol was added to the deposit and the cells were disrupted by vigorous shaking. The ethanol extract was separated from the cells by centrifugation at 10,000rpm for 5mins. The extraction procedure was repeated until the cells were completely bleached, and all the supernatants were collected as crude violacein for measuring the violacein concentration. The absorbance of ethanol solution of crude violacein sample was measured by using ultraviolet-visible spectrophotometer (Beckman DU800, USA) (extinction coefficient of violacein = 10.955L/(gcm) in ethanol at 570nm) [18].

Optimization for the Production of Violacein

Effect of Various Media on Violacein Production: For optimization of the media for enhancing the yield of violacein, 1ml of overnight culture of *C. violaceum* was inoculated into three different media such as Nutrient broth (NB), Brain heart infusion broth (BHI) and peptone broth (PB). The culture

flasks were incubated at 37°C for 48hr. After incubation, the broth was taken for pigment extraction, estimation as stated above [19,18].

Effects of Temperature on Violacein Production: 1ml of overnight culture of *C. violaceum* was inoculated into nutrient broth and incubated at different temperature for 24 hours. The fermentation was carried out at 25°C, 30°C, 37°C, 45°C and 52°C at the end of the incubation the optimum temperature for pigment production was observed [20].

Effects of Incubation Period on Violacein Production: 1ml of overnight culture of *C. violaceum* was inoculated into nutrient broth and incubated at different incubation period, the fermentations were carried out for different time and the durations were from 12hr to 92hr at an interval of 12hr. The optimum incubation period for pigment production was observed [20].

Effects of pH on Violacein Production: The isolate was inoculated into nutrient broth and kept at different pH (4-9 at an interval of 0.5) for 72hr incubation. The production of the pigments was estimated after incubation. The maximum pH, at which the maximum production of violacein takes place, was observed [18].

Effects of Different Carbon Sources on Violacein Production: 1ml of overnight culture of *C. violaceum* was supplemented with different carbon sources at 5% concentration to study their effect on the pigment production. The carbon sources chosen in the present study were glucose, fructose, maltose, lactose, sucrose, dextrose and starch. After incubation in an optimal condition, the violacein was quantified [20].

Effects of Different Nitrogen Source on Violacein Production: 1ml of overnight culture of *C. violaceum* was supplemented with different nitrogen sources at 5% concentration. Ammonium nitrate, ammonium chloride, yeast extract, beef extract and peptone were chosen to evaluate the effect of different nitrogen sources on violacein production [20].

Assay of Antibacterial Activities of Violacein: Antibacterial activities of the crude violet pigment were carried out using Kirby Bauer's disc diffusion method [21].

Preparation of Violacein Disc: Whatman filter paper was perforated with a perforator and wrapped in an aluminum foil then put in a container with lid and sterilized by autoclaving at 121°C for 15mins. A one in two dilution of the violacein was done at different concentrations (35, 17.5, 8.8, 4.4 and 2.2) mg/ml. the concentrations were used to impregnate the disc and allowed to dry.

Antibacterial Activity Testing

(Kirby-Bauer Disc Method): Using a sterile wire loop, 3-5 colonies of the isolates were emulsified in 4ml Nutrient broth and incubated for 6 hours at 37°C. The turbidity of the test suspensions were matched with 0.5ml Mcfarland turbidity standard which is roughly equivalent to 150million cells/ml. A sterile cotton swab was dipped into the standardized bacteria test suspension and used to evenly inoculate the entire surface of the Nutrient agar plates. After the agar surface has dried for about 5minutes, the violacein disc of different concentration was placed on them, using a sterile forcep. Then plates were incubated for 24 hours at 37°C, after which they were examined for zones of inhibition. The zones were measured with graduated ruler and recorded in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC): A one in two dilutions of violacein were carried out in the tubes using nutrients broth as diluent to obtain different concentrations of violacein, (35, 17.5, 8.8, 4.4. and 2.2). The concentration of violacein used was 3500µg/ml=35mg/ml. Test organism was inoculated into each tube and the tubes were incubated at 37°C for 2hours. After incubation, the tubes were examined for growth. The MIC was reported as the lowest concentration that showed no growth.

| Sample | Growth on NA | Gram stain | Cat | Ox | Mot | MR/VP | Indole | Cit | Urease | Gelatin | Glu | Gal | Lac | Malt | Fru | Ara | Growth on KIA |
|---------------|---|------------|-----|----|-----|-------|--------|-----|--------|---------|-----|-----|-----|------|-----|-----|---------------------|
| Otamiri River | Rounded convex smooth rod 0.6-0.9µm violet colony | - ve | + | + | + | -/- | - | + | - | + | + | - | - | - | - | - | No H ₂ S |

Keys: NA: Nutrient Agar; Ox: Oxidase; Mot: Motility; MR: MethylRed; Cat: Catalase test; VP: Vogues Proskauer; Cit: Citrate utilization; Glu: Glucose; Lac: Lactose; Malt: Maltose; Fru: Fructose; Ara: Arabinose; KIA: Kliger; Iron Agar --- Negative; + -- Positive

Table 1: Morphological and Biochemical characteristics of bacterial Isolates from Otamiri River.

Quantification of Violacein Produced

| Isolate | Source | Violacein produced (mg/l) |
|---------------------|---------------|---------------------------|
| <i>C. violaceum</i> | Otamiri river | 34.96 |

Table 2: Quantification of violacein produced.

The violacein produced was extracted and quantified using spectrophotometer. The concentration of violacein produced was 34.96mg/l.

Optimization for the Production of Violacein

Violacein produced was optimized under different culture conditions.

Effect of Media on Violacein Production: Selection of suitable media was necessary for the production of violacein. Among the three media used as shown in Table 3, Nutrient

Statistical Analysis: The data from this study were analyzed using one way ANOVA statistical techniques or tools. This was used to analyse the data collected during the course of this survey with a simple bar chart. Mean standard deviation was used in the antibacterial susceptibility test.

Results

Morphological and Biochemical Characteristics of Bacterial Isolates

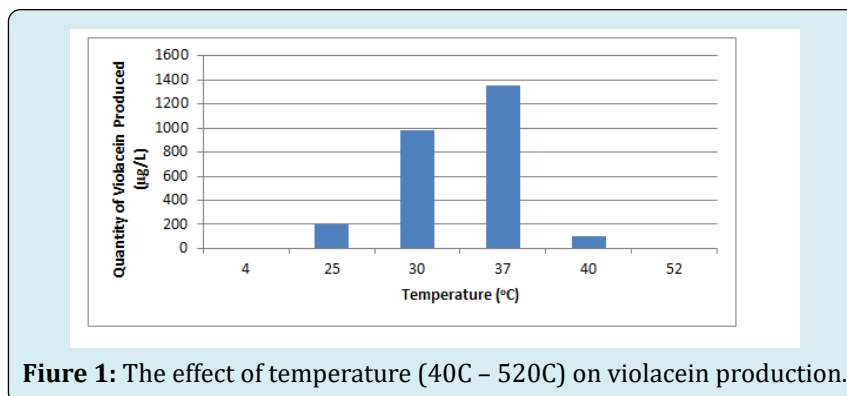
The Isolate obtained from Otamiri River was differentiated on the basis of its cellular morphological, and biochemical tests. Table 1 show that the isolate was 0.6 – 0.9µm violet/purple, round, convex smooth colonies, showing Gram-negative rods, positive for catalase, oxidase, motility, and citrate utilization and liquefies gelatin, produces hydrogen cyanide. It was urease, Methyl Red, Vogues Proskauer and Indole negative. Carbohydrate fermentation tests revealed that the isolate possesses the ability to ferment glucose only. Growth on Kliger Iron Agar (KIA) showed alkaline/acid with no H₂S production. These results obtained were compared with Bergey's Manual of determinative bacteriology and the isolate was identified as *C. violaceum* Table 2.

broth showed the highest pigment production (1000µg/l) followed by peptone broth (500 µg/l) while Brain Heart Infusion (BHI) showed lower production of pigment (400µg/l).

| Media | Violacein produced (µg/l) |
|----------------------------|---------------------------|
| Nutrient Broth | 1000 |
| Brain Heart Infusion (BHI) | 400 |
| Peptone Broth | 500 |

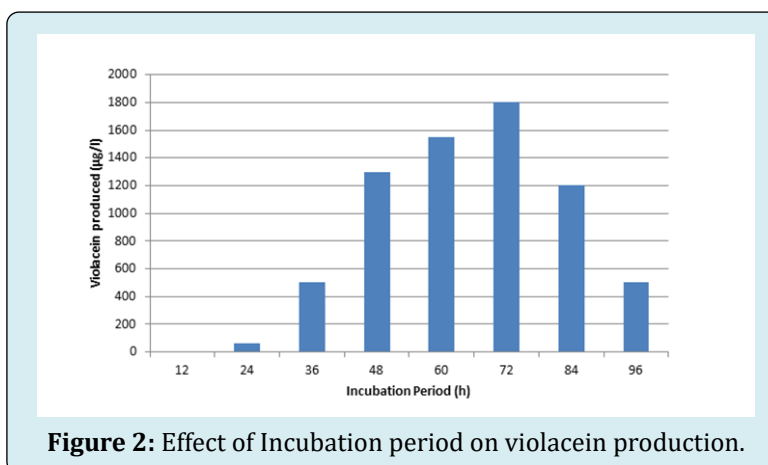
Table 3: Effect of Culture Media on violacein produced.

Effect of Temperature on Violacein Produced: Temperature is a critical factor in the production of violacein. Figure 1 shows the effect of temperature (4°C – 52°C) on violacein production, the result reveals that violacein production was maximum at 37°C. Increase in temperature above 37°C resulted in gradual decrease in violacein production.



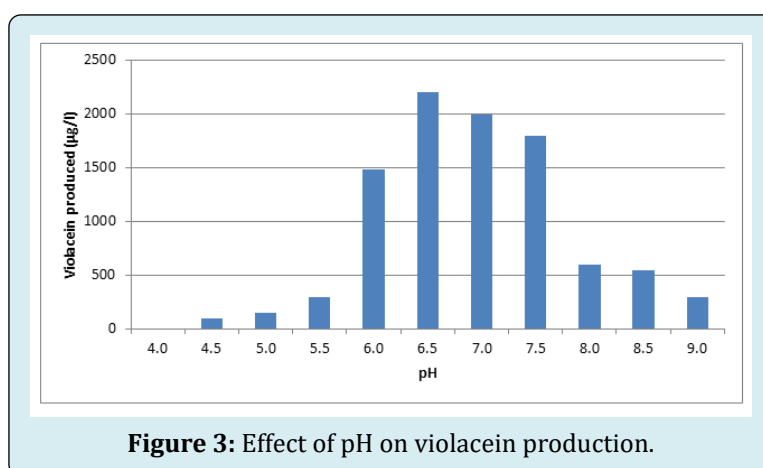
Effect of Incubation Period on Violacein Produced: Violacein production was determined during different period of incubation (12, 24, 36, 48, 72, 60, 84, 96 hours).

Figure 2 shows that the violacein production was highest at 72hr (1800µg/l) after which the production declined.



Effect of pH on Violacein Produced: Violacein production at different pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0) was examined. Figure 3 shows that violacein production was clearly affected by initial pH 4.0. The maximum pH

for violacein production was observed at 6.5 (2200µg/l). As the pH increased above 6.5, the production of violacein decreased.



Effect of Carbon Sources on Violacein Produced: Growth of microorganism and the production of metabolites are

influenced by the organism's utilization of carbon sources during fermentation. The growth medium was inoculated

with 5% different carbon sources (dextrose sucrose glucose, maltose, lactose fructose and starch). Glucose was the best carbon source for violacein production as it yielded high

amount of violacein (2300 $\mu\text{g/l}$) followed by dextrose and maltose. The least was starch (Figure 4).

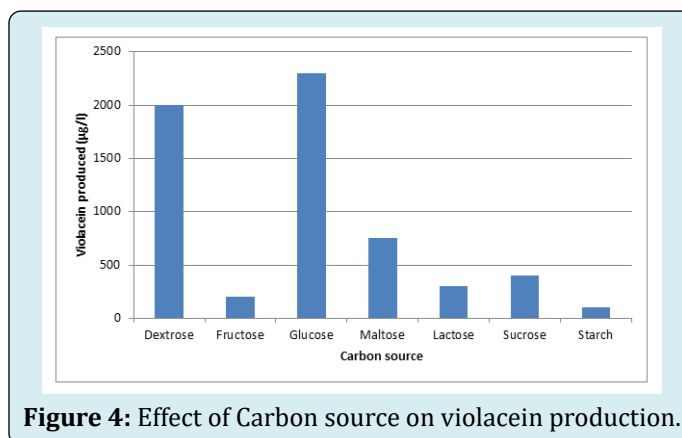


Figure 4: Effect of Carbon source on violacein production.

Effect of Nitrogen Source: Various nitrogen sources were examined for their effects on violacein production by the isolate (*Chromobacterium violaceum*). As shown on Figure 5, organic nitrogen sources were found to yield more violacein

than the inorganic. Yeast extract was found to be the most efficient nitrogen source (2500 $\mu\text{g/l}$) followed by beef extract (1800 $\mu\text{g/l}$) and Peptone (1000 $\mu\text{g/l}$).

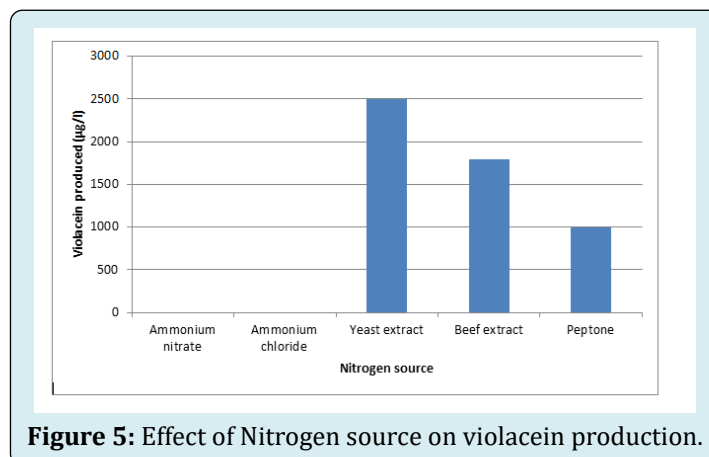


Figure 5: Effect of Nitrogen source on violacein production.

Antibacterial Susceptibility

The result of antibacterial sensitivity test (Table 4 & 5) shows that *Staphylococcus aureus* and *Escherichia* were

highly sensitive to violacein at the concentration of 4.4 while *Klebsiella aerogenes* and *Pseudomonas aeruginosa* were resistant to violacein.

| Test Organism | 35 | 17.5 | 8.8 | 4.4 | 2.2 | Negative Control (Ethanol) | Positive Control (Ciproflox) |
|-------------------------------|------------------|-------------------|------------------|------------------|-----------------|----------------------------|------------------------------|
| <i>S. aureus</i> | 26 \pm 5.66 | 17.50 \pm 10.61 | 17.00 \pm 4.24 | 15.00 \pm 7.07 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 22.50 \pm 3.54 |
| <i>E. coli</i> | 30 \pm 7.07 | 20.00 \pm 0.00 | 14.50 \pm 0.71 | 17.00 \pm 1.41 | 9.00 \pm 1.41 | 0.00 \pm 0.00 | 26.50 \pm 4.95 |
| <i>Klebsiella aerogenes</i> | 14.00 \pm 8.49 | 8.00 \pm 2.83 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 11.50 \pm 4.95 |
| <i>Pseudomonas aeruginosa</i> | 0.0 \pm 0.0 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 0.00 \pm 0.00 |

Violacein Concentration (mg/ml) and Zone Of Inhibition (mm).

Table 4: Antibacterial Susceptibility Test.

Minimum Inhibitory Concentration of Violacein (MIC)

| Test Organism | 35 | 17.5 | 8.8 | 4.4 | 2.2 | Negative Control (Ethanol) | Positive Control (Ciproflox) |
|-------------------------------|----|------|-----|-----|-----|----------------------------|------------------------------|
| <i>S. aureus</i> | - | - | - | - | + | - | - |
| <i>E. coli</i> | - | - | - | - | + | - | - |
| <i>Klebsiella aerogenes</i> | + | + | + | + | + | - | + |
| <i>Pseudomonas Aeruginosa</i> | + | + | + | + | + | - | + |

(-) No visible turbidity (No growth)

(+) Visible turbidity (growth)

The minimum inhibitory concentration (MIC) of violacein was examined and the result showed that the MIC ranged from 35-4.4mg/ml.

Table 5: Minimum Inhibitory Concentration of Violacein (MIC).

Discussion

Chromobacterium violaceum was isolated from Otamiri River in Owerri, Imo State. The isolate is a facultative anaerobe, motile, Gram-negative bacillus. *Chromobacterium violaceum* has attracted much attention due to its bioactive pigment violacein which is a secondary metabolic [22]. Investigation on the quantification of violacein production by *Chromobacterium violaceum* isolated from Otamiri River in Owerri showed that 35mg/ml of violacein was produced at 570nm and this agrees with the work of Gallardo, et al. [23]. Violacein production is an important property of *Chromobacterium violaceum* so the optimization conditions for violacein production such as growth media, temperature, incubation period, pH, and Carbon and Nitrogen sources are a prerequisite. In the present work, an attempt was taken to analyze the effect of media on violacein production, among the three media used, Nutrient broth resulted in a favourably high violacein production of 1000mg/l, followed by Peptone broth (5000mg/l), Brain Heart infusion showed lower production of violacein (4000mg/l). It corroborates with the work of Ahmed, et al. [19], Vishnu and Palaniswamy, et al. [22]. Nutrient broth gave maximum production of violacein, may be due to the capacity of the isolate to utilize tryptone and yeast extract. In BHI absence of tryptone, peptone and yeast extract may lead to lower production. Temperature has got profound effect on the production of violacein. The effect of temperature in the range of 4°C – 52°C was examined on the violacein production by *C. violaceum*, there was no growth at 4°C -52°C, the optimal temperature was found to be at 37°C with maximum production of 135mg/ml. In other studies with violacein producing bacteria, the optimal temperature was reported as 28°C for *Chromobacterium violaceum* BB-78 [24], 33°C for *Chromobacterium violaceum* CCT 3496 25°C for *Pseudoalteromonas* spp X 82144 [25] and 20°C for *Dunganelle* spp DSM 1953 [18], respectively. Zhang, et al. [26] studied the optimization conditions for violacein production by recombinant *Citrobacter freundii*, the maximum violacein production was at 37°C, and this result

is similar to our present study. Temperature is one of the most important environmental factors affecting the growth of micro-organism.

Incubation time plays a substantial role in the maximum pigment production. The influence of incubation period in violacein production by *C. violaceum* was studied at interval of 12hr, we observed that the pigment production was increased with respect to high incubation time, from 24hr to 72hr and after, the production declined. The maximum production of violacein (1800µg/l) was observed at 72hr incubation, the lowest production was observed at 24hr of incubation, no production at 12hr incubation. Wang, et al. [18] reported that the maximum production of violacein was at 32hr by a new strain *Dunganelle* spp B2. Sivaranjini [27] reported the maximum production of violacein at the third day of incubation by *C. violaceum* JC1 in solid state fermentation and Vishnu and Palaniswamy, et al. [22] reported the maximum production of violacein to be at 72hr of incubation. In Mendes, et al. [20] the highest crude violacein production was obtained after 36hr which *C. violaceum* CCT3496 and after 40hr for recombinant *Citrobacter freundii* ATCC05417 in the liquid cultures. Ahmed, et al. [19] reported the maximum production of violacein was at 4th hour after bacterial growth that was at the late exponential phase. Their results suggested that the pigments produced by bacteria are secondary metabolites [28].

The pH of the growth medium plays an important role by inducing morphological changes in microbes. The maximum pH may affect cell membrane function, cell morphology and structure, solubility of salts, the ionic state of substrate, uptake of various nutrient and production biosynthesis. In general, cells can only grow within a certain pH range and metabolite formation is also affected by pH [29]. The pH change observed during the growth of microbes also affects the product stability in the medium [30]. Violacein production at various pH (4.0 – 9.0) was studied and we observed that the maximum pigment production was obtained at pH 6.5.

As the pH increased from 4.5 to 6.5, the pigment production increased. The production was found to decrease beyond pH 7.0. Similar results were obtained in a study of Zhang, et al. [26]. The growth and metabolic production of microorganism are influenced by the organism's utilization of carbon and nitrogen sources during fermentation. The influence of carbon and nitrogen sources on the pigment production was observed in this study and glucose (2300µg/l) was found to be the optimum carbon source for violacein production followed by dextrose (2000µg/l) and starch was the least (100µg/l). Drew and Demain, et al. [31] reported that the use of carbon sources resulted in a decrease cellular growth and an increase in the secondary metabolite production. Yeast extract was found to be the best nitrogen source for pigment production in our study, whereas ammonium nitrate and ammonium chloride strongly inhibited violacein production. Our results agree with the result of Vishnu and Palaniswamy, et al. [22]. Antibacterial potentials of violacein were investigated in our study and we discovered that *Staphylococcus aureus* and *Escherichia coli* were highly sensitive to violacein.

Staphylococcus aureus has the highest concentration at 35mg/ml and the lowest at 4.4mg/ml with zone of inhibitions of 26 and 15 (mm) respectively. *Klebsiella aerogenes* has high concentration with zone of inhibition of 14mm in diameter while *Pseudomonas aeruginosa* was resistant to all concentration. This is in agreement with the work done by Cazoto, et al. [32]. The Minimum Inhibitory Concentration (MIC) of violacein against different organisms was examined and it was discovered that the MIC of violacein was 4.4 against *S. aureus* and *E.coli*. *Klebsiella aerogenes* and *Pseudomonas aeruginosa* has no inhibitory activity. It corroborates with work done by Subramanian, et al. [10] who discovered MIC for *Staphylococcus aureus* at 5.7 and resistant to *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The minimum bacteriocidal concentration of *Staphylococcus aureus* and *Escherichia coli* was 8.8mg/ml while the zone of inhibition on plate and the MIC observed at 4.4mg/ml for *Staphylococcus aureus* and *Escherichia coli* may be considered as bacteriostatic. The negative controls (Ethanol) did not show any bacteriocidal effect against the test organism on the other hand the positive control (Ciprofloxacin) showed bacteriocidal effect against the aforementioned organisms.

Conclusion

The result of this research has demonstrated the antibacterial potentials of violacein obtained from *Chromobacterium violaceum*. A significant increase in violacein production was obtained when the organisms was grown at 37°C, pH 6.5 for 72 hours in the presence of glucose as carbon source and yeast extract as nitrogen source. The successful inhibition of both Gram-positive organism

(*Staphylococcus aureus*) and Gram-negative organism (*Escherichia coli*) by violacein depicts it as a potential source for the production of broad spectrum antibiotics, which can be used to treat some infections.

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