World Journal of Advanced Pharmaceutical and Medical Research

Journal homepage: https://zealjournals.com/wjapmr/

ISSN: 2799-0656 (Online)

(RESEARCH ARTICLE)

Check for updates

Assessment of antibacterial potentials of violacein extract from *Chromobacterium violaceum* isolated from domestic and recreational water sources in Owerri, Nigeria

Lovelyn Chinyeaka Ugenyi, Joy Nkeiruka Dike-Ndudim, Henry Chidozie Amah and Chizaram Winners Ndubueze *

Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria.

World Journal of Advanced Pharmaceutical and Medical Research, 2021, 01(02), 007–012

Publication history: Received on 09 July 2021; revised on 02 September 2021; accepted on 04 September 2021

Article DOI: https://doi.org/10.53346/wjapmr.2021.1.2.0024

Abstract

This study was carried out with the aim of assessing the antibacterial potentials of violacein extracted from *Chromobacterium violaceum* isolated from domestic and recreational water sources in Owerri, Nigeria. Water samples were collected from different locations of the domestic water sources, five different swimming pools, and three borehole stations using sterile amber bottles. The isolation of *C. violaceum* was done using pour plate method on nutrient agar. The violet colonies of *C. violaceum* were counted, characterized and identified using standard microbiological and biochemical techniques. The mean viable bacterial counts were high. Water sample from Otamiri station-1 have the highest bacterial count (200×10^1 CFU/ml and 19.50×10^1 CFU/ml) respectively. Swimming pool 1 and 3 bacterial counts were (4.50×10^1 CFU/ml, 11×10^1 CFU/ml and 11.50×10^1 CFU/ml) respectively. For borehole 1, 2 and 3, swimming pool 2, 4 and 5, counts were (0.00×10^1 CFU/ml). Ethanolic extraction of violacein from *C. violaceum* was performed from a 48-hour culture broth. The sensitivity of the bacteria isolates to violacein was assayed on nutrient agar and nutrient broth by agar diffusion and broth dilution methods respectively. All the bacterial isolates were susceptible to the violacein extract at various concentrations, except MRSA that showed resistance to the violacein at 2.19mg/ml for extract from recreational water isolate and at 17.5mg/ml to 2.19mg/ml for extract from domestic water isolates. Conclusively, violacein has the potential to be used as an antibacterial compound for treatment of multidrug resistant bacterial infections.

Keywords: Violacein; *Chromobacterium violaceum*; Antibacterial; Extract; Water Sources

1. Introduction

The increasing rate of microbial resistance to a number of antimicrobial agents is becoming a major public health problem. The increasing use and misuse of existing antibiotics in human, veterinary medicine in agriculture has further aggravated the problem [1]. Common species among them are methicillin-resistant *Staphylococcus aureus* (MRSA), Penicillin-resistant *Streptococcus pneumoniae,* vancomycin-resistant *Enterococcus* and *Mycobacterium tuberculosis* [2, 3]. It is further stated that about 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment [1]. Thus there is an urgent need for new classes of antimicrobial compounds to overcome existing resistance mechanisms and to effectively combat these human pathogens that can cause life threatening infections. The first antibiotic from marine bacterium was identified and characterized in 1966 [4].

The violacein is a natural purple, blue pigment extracted from various Gram-negative bacteria including *Chromobacterium violaceum, Collimonas* sp., *Duganella* sp., *Lodobacter fluviatile, Janthinobacterium lividum,* and *Microbulbifer* sp. Violacein exhibits many biological properties such as broad-spectrum antimicrobial, antiviral, antiprotozoal, and antioxidant activities, and exerts significant cytotoxicity toward several tumor cell lines. Various

* Corresponding author: Chizaram Winners Ndubueze
Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria.

biological and pharmacological properties of violacein have made it an attractive tool for medical and biotechnological research [5].

Violacein is a natural purple pigment found in microorganisms, especially in *Chromobacterium violaceum* living in unique environments, from the tropic to glaciers. It acts as natural defense system against other organisms, including: Amoebas, bacteria and parasites. As an antibacterial agent, violacein showed activity against *Mycobacterium tuberculosis* [6].

The pigmented molecule is of particular interest and understanding violacein's function and mechanism of action has relevance to those unmasking any of its commercial or therapeutic benefits. Unfortunately, the production of violacein and its related derivatives is not easy and so various groups are also seeking to improve the fermentative yields of violacein through genetic engineering and synthetic biology [7].

Violet-pigmented bacteria, which have been described since the end of the 19th century, are occasionally the causative agent of septicemia and sometimes cause fatal infection in human and animals. Bacteria, producing violet colonies due to the production of a non-diffusible pigment violacein, were classified as a redefined genus, *Chromobacterium* [8].

Chromobacterium violaceum is a Gram negative bacteria found in water and soil samples from tropical and subtropical regions of the world. Due to its biotechnological potential, *C. violaceum* had its genome sequenced by the Brazilian National Genome Project. The most notable characteristic of *C. violaceum* is the production of the chemically well characterized pigment named violacein. Previous studies indicated antibiotic and antichagasic, antitumoral, and antileishmanial activities of violacein [9, 10, 11]

It is part of the normal flora of water and soil of tropical and subtropical regions of the world. It grows readily on nutrient agar, MacConkey agar and blood agar at 35-37OC, producing distinctive smooth low convex colonies with a dark violet colonies with beta-hemolysis. Some strains of the bacteria which do not produce this pigment have also been reported, which may make diagnosis even more difficult.

2. Material and methods

2.1. Study Area and Water Sources

This study was carried out in Owerri, Imo State, Nigeria. Owerri is the capital city of Imo State in Nigeria, set in the heart of Igbo land. It is bordered by two different domestic water sources: Otamiri River to the east and Nworie River to the south. Owerri has a tropical wet climate according to the koppen-Geger system. Rain falls for most months of the year with a brief dry season. Population of Owerri is 215038 people, latitude of 57500 (5450.00"N), longitude of 71167 (77'0.012"E) and Altitude of 152. Inadequate supply of portable drinking and domestic water in Owerri has led to the installation of underground and overhead water tanks. Also, borehole is installed from the supply of underground water. Owerri has a lot of hotels and due to its location, people from different parts of Nigeria visit the city. In each of these hotels is a swimming pool for recreation of the hotel inmates and other persons in the neighborhood [12].

Recreational use of water has important benefits to health and wellbeing of humans. Yet, there may also be adverse health effects associated with it, if the water is polluted or unsafe [13]. Common sources of contamination in swimming pool water quality include the water source, bather-derived chemicals, and pool maintenance chemicals [14]. These are evidence that *C. violaceum* can be isolated from domestic and recreational water sources.

2.2. Sample Collection

Three water samples were collected from different locations of the Otamiri River and five water samples from five different swimming pools of five hotels, and three from different boreholes in Owerri using sterile amber bottles. The isolation *C. violaceum* was carried out by pour plate method on nutrient agar. The standard methods for the isolation and identification of bacteria as described by Dike-Ndudim *et al.,* [12] was adopted in the analyses. Ten (10) millimeter of the water samples was aseptically transferred into 250ml Erlenmeyer flask containing 22.5ml nutrient broth medium followed by incubation at 30°C for 24 hours. One loopful of bacterial culture was subcultured onto nutrient agar plates and incubated at 30°C for 24 hours. Serial sub-culturing was carried out until single bacterial colonies were obtained. The isolates from the contaminated water which appeared violet in colour were characterized to confirm *Chromobacterium violaceum*.

2.3. Extraction of Violacein

Compound-violacein was extracted from *C. violaceum* and purified as described by Renee and Kendall, [15] with little modification. Ethanolic extraction of violacein was performed from a 48 hour broth culture.

2.4. Disc Preparation and Susceptibility Test

The sensitivity of the test bacteria to violacein was assayed on nutrient agar and nutrient broth media by agar diffusion and broth dilution methods respectively. Kirby-Bauer disc diffusion method was used to determine the antibacterial activity of the crude violacein extracts as follows (Harley-Prescott, 2002). Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, P. aeuginosa,* and *E. faecalis* were sub-cultured into Nutrient broth medium and incubated at 37°C for 12 hours. Then, 0.1 ml of the broth culture was inoculated onto nutrient agar plates in duplicates. The plaates were allowed to air dry for five minutes before applying the antibiotic discs which were whatman filter papers (wet strengthened, 0.7mm in diameter), previously impregnated with 50µl of the crude ethanol extract. 5µg/ml ciprofloxacin was used as the antibiotics standard. The zones of growth inhibition were determined after 18 hours of incubation at 37°C for the violacein extracts by measuring the diameter of the cleared area with a meter rule. Strain resistance was determined using the latest breakpoint tables available at the Clinical Laboratory Standards Institute [16].

2.5. Minimum Inhibitory Concentration

The test tubes which were already containing various concentration of violacein extract (ranging from 35mg/ml to 2.19mg/ml) for both domestic and recreational water isolates were inoculated with 50µl of the inoculums suspensiom using a micropipette. Tubes were sealed and incubated at 37°C for 18-22 hours. The positive control drug was run in parallel with the violacein extract. All plates were inoculated in duplicates. The Minimum Inhibitory Concentration (MIC) was read as the lowest concentration without visible growth.

3. Results

The mean viable bacterial counts from the water sources were generally high. Counts from domestic water source station 1 and 3 were highest with 20.00×10^1 CFU/ml and 19.50×10^1 CFU/ml, respectively. Bacterial counts recorded for domestic water sample 2, recreational water samples 1 and 3 were 14.50×10^1 CFU/ml, 11×10^1 CFU/ml and 11.50×10^1 CFU/ml, respectively. Fore borehole 1, 2, and 3, swimming pools 2, 4 and 5, counts were 0×10^1 CFU/ml. Otamiri River has more bacterial contamination than the recreational water samples as expressed by their viable bacterial counts.

Table 1 Mean zones of inhibition for the swimming pool extract dilutions in relation with the positive control ciprofloxacin 5mg/ml and negative control alcohol 95% on the bacterial isolates

	Zones of inhibition (mm)			
Concentration of extracts (mg/ml)	**MRSA**	***E. coli***	***P. aeruginosa***	***E. faecalis***
35.0	21.50	29.50	27.50	26.50
17.5	19.00	26.00	25.50	25.00
8.75	17.00	24.50	23.50	22.50
4.38	15.00	21.00	20.50	21.00
2.19	14.00	16.00	18.00	18.00
Cipro (5mg/ml)	39.50	36.50	40.00	29.50
Alcohol	0.00	0.00	0.00	0.00

The bacterial isolates used for this study showed high resistance to conventional antibiotics used on them.

Table 1 shows that the positive control had more significant zones of inhibition in its effect on all bacteria. Also, both extracts at 35mg/ml showed a sensitive zone (significant zones) of inhibition for all the organisms. At 17.5mg/ml and 8.75mg/ml, all were still sensitive except for MRSA which showed resistance at 17.5mg/ml (9mm) and below. Even up to the concentration of 4.38 and 2.19mg/ml, *E. coli* (21mm and 17mm) and *P. aeruginosa* (20mm and 16.5mm) were

still sensitive as shown in the figure 4.2 below. From figure 4.3, MRSA was sensitive from 35mg/ml to up to 4.38mg/ml in a decreasing linear trend with zones of inhibition ranging from 21.5mm to 15mm.

E. coli P. aeruginosa and *E. faecalis,* showed sensitivity in all levels of the dilutions of the extract, from 35mg/ml to 2.19mg/ml with zones of inhibition decreasing in a linear trend as shown in table 2 below. In both table 1 and 2, the positive control showed a significantly high zones of inhibition against the selected organisms with the negative control showing no effect on the selected bacteria.

Table 2 Mean zones of inhibition for the Otamiri extract dilutions in relation with the positive control ciprofloxacin 5mg/ml and negative control alcohol 95% on the bacterial isolates

	Zones of inhibition (mm)			
Concentration of extracts (mg/ml)	**MRSA**	***E. coli***	***P. aeruginosa***	***E. faecalis***
35.0	21.50	31.50	25.00	38.00
17.5	9.00	26.00	23.00	35.50
8.75	0.00	24.50	20.50	26.00
4.38	0.00	21.00	20.00	10.00
2.19	0.00	17.00	16.50	9.00
Cipro (5mg/ml)	40.50	36.50	39.50	28.50
Alcohol	0.00	0.00	0.00	0.00

The MIC and Minimum bactericidal concentration (MBC) of the *C. violaceum* extract from domestic and recreational water sources on some selected bacteria in relation to ciprofloxacin was done.

Table 3 MIC and MBC values of violacein produced by *C. violaceum* isolated from Otamiri against test organisms

Isolates	**Ciprofloxacin (5µg/ml) (Positive control)**		**Ethanol (Negative control)**		**Concentration of violacein extracts (mg/ml)**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
MRSA	2.50	5.00	0.00	0.00	17.50	17.50
E. faecalis	0.62	1.25	0.00	0.00	8.75	8.75
E. coli	2.50	2.50	0.00	0.00	8.75	8.75
P. aeruginosa	0.620	1.25	0.00	0.00	8.75	8.75

Table 4 MIC and MBC values of violacein produced by *C. violaceum* isolated from swimming pool against test organisms

Isolates	**Ciprofloxacin (5µg/ml) (Positive control)**		**Ethanol (Negative control)**		**Concentration of violacein extracts (mg/ml)**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
MRSA	1.25	2.50	0.00	0.00	8.75	17.50
E. faecalis	0.62	1.25	0.00	0.00	17.50	17.50
E. coli	2.50	2.50	0.00	0.00	8.75	8.75
P. aeruginosa	0.62	1.25	0.00	0.00	8.75	8.75

4. Discussion

In this study, we isolated *C. violaceum* from domestic and recreational water sources, which is in line with the report by Dike-Ndudim *et al.,* [12]. No *C. violaceum* was isolated from the three (3) borehole water samples, this contrasts the same result by Dike-Ndudim *et al.,* [12]. This may be attributed to the size and sites of borehole water samples as compared to those assessed by the researcher. *C. violaceum* was also isolated from two recreational water samples. For identification of *C. violaceum,* the result of this study agrees with the report by Ahmad *et al.,* [17]. The isolate showed morphological characteristics as gram negative rod similar to that isolated by Ahmad *et al.,* [17].

In this study, the violacein extract obtained from the *C. violaceum* assessed had similar effect as the one in previous study by Anju *et al.,* [18]. The extract from both domestic and recreational water isolates showed similar trend in result for their zones of inhibition.

E. faecalis and MRSA, the Gram-positive bacteria showed high sensitivity to the violacein at concentrations 17.5mg/ml and 8.75mg/ml respectively. The same concentration led to the loss in viability of these bacteria after 24 hours as determined by the MBC regardless of their multidrug resistance. This result is corroborating the report of Cazoto *et al.,* [7] which showed activity of violacein extract against human *Staphylococcus aureus* at concentration of 17μM and loss of viability at final concentration of 15μM, or approximately 5mg/L. *P. aeruginosa,* and *E. coli,* the Gram negative bacteria were also susceptible to the violacein extract at concentrations of 8.75mg/ml respectively. Studies by Aranda *et al.,* [19], highlighted the tendency of Gram-positive strains to be more susceptible to violacein than Gram negative strains. They attributed their findings to the presence of the outer membrane within these bacteria which provides protection against violacein, which was opposite to our findings.

The commercial antibiotics ciprofloxacin (positive control) showed effect on all the bacterial isolates. The violacein showed activity similar to the standard ciprofloxacin but the effects of the standard ciprofloxacin were higher than that of violacein on agar disc diffusion assay.

5. Conclusion

Several investigations have been made on the isolation of *C. violaceum* from water sources. In this study, *C. violaceum* was isolated from domestic and recreational water sources in Owerri. Characterization and identification of the isolate match the results to the reports of match the results to the reports by previous authors. For this study, the isolate showed violet pigmented colonies on nutrient agar plate. Extraction of violacein from the isolate was also recorded in our study. The violacein extract also shows similar effect as reported in previous studies. The marine bacterial isolate, *Chromobacterium violaceum* was found to produce significant antibacterial pigment which was confirmed as violacein. It inhibited a series of test bacteria isolates tested on them, both Gram positive and Gram negative were susceptible to it. The violacein showed good antibacterial activity as compared to standard ciprofloxacin against bacteria and isolates. This study illustrates the potential of violacein pigment isolated from *Chromobacterium violaceum* as an antibacterial agent for MRSA and other pathogenic bacteria. Therefore, *Chromobacterium violaceum* violacein can be further purified and used for the production of novel novel antibiotics for the treatment of all kinds of MDR *Staphylococcus aureus,* Urinary tract infections and MDR *Streptococcus* infections.

Compliance with ethical standards

Disclosure of conflict of interest

There is no Conflict of interest.

References

[1] Todar K. The Good, the bad, and the Deadly. Science Magazine. 2004; 1421.

[2] Barrett CT, Barrett JF. Antibacterial: are the new entries enough to deal with the emerging resistance problems". Current opinion Biotechnology. 2003; 14(6): 621-626.

[3] Dancer ST. The effect of antibiotics on methicillin-resistant Staphylococcus aureus. Journal of Antimicrobial Chemotherapy. 2008; 61: 246-253.

[4] Burkholder PR, Pfister RM, Leitz FH. Production of a pyrrole antibiotic by a marine bacterium. Applied Microbiology. 1966; 14: 649-653.

[5] Wang H, Wang F, Zhua X, Yan Y, Yua X, Jiang P. Biosynthesis and Characterization of Violacein, Deoxyviolacein and Oxyviolacein in Heterologous Host, and their Antimicrobial Activities". Biochemical Engineer Journal. 2012; 67: 148-155.

[6] Nelson D, Giselle ZJ, Carmen VF, Patricia M. "Violacein: properties and biological activities". Biotechnology and Applied Biochemistry. 2007; 48: 127-133.

[7] Cazoto LL, Martins D, Ribeiro MG, Duran N, Nakazato G. Antibacterial activity of violacein against Staphylococcus aureus isolated from Bovine Mastitis". Journal of antibiotics. 2011; 64: 395-397.

[8] Nelson D, Carlos FMM. Chromobacterium violaceum: A Review of Pharmacological and Industrial Perspectives. Critical Reviews in Microbiology. 2001; 27(3): 201-222.

[9] Duran N, Menck FM. Chromobacterium violaceum: A Review of Pharmacological and Industrial Perspective. Critical Review of Microbiology. 2011; 27: 201-222.

[10] Melo PD, Maria SS, Vidal BD, Haun M, Duran N. Violacein cytotoxicity and Induction of apoptosis in V79 cells. In vitro cellular and Developmental Biology – Animal. 2000; 36(8): 539-543.

[11] Leon LL, Miranda CC, de Souza AO, Duran N. Antibacterial activity of the violacein extracted from Chromobacterium violaceum. Journal of Antimicrobial Chemotherapy. 2001; 48(3): 449-450.

[12] Dike-Ndudim JN, Dike DO, Ukogo I, Egbuobi RC, Nwigwe HC, Iwuala MOE. Bacteriological and Physico-chemical qualities of borehole water in Imo State". Microbes in the spotlight: Recent progress in the understanding of Beneficial and Harmful Microorganisms. Foratex Research Center. Florida USA: Brown Walker Press Boca Raton. 2016; 37-39.

[13] Akhionbare SMO. The Environment. M C Computer Press, Nnewi, Nigeria. 2009; 193: 177.

[14] Jmaiff B, Lindsay K, Wang W, Vemula S, Jaeger BT. Sweetened swimming pools and hot tub. Environmental Science and Technology Letters. 2017; 4(4): 149.

[15] Reene SB, Kendall MG. Extraction of Violacein from Chromobacterium violaceum provides a new quantitative bioassay for N-acyl homoserine lactone autoinducers". Journal of Microbiological Method. 2000; 40: 47-55.

[16] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-18. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.

[17] Ahmad WA, Wan Ahmad WY, Zakaria ZA, Yusof ZN. Isolation of Pigment-producing Bacteria and Characterization of the extracted pigments, Application of Bacterial Pigment as Colorant. Springler briefs in Molecular Science. 2012.

[18] Anju S, Nishanth SK, Krishnakumar B, Dileep Kumar BS. Combination of Violacein and Azoles that leads to Enhanced Killing of Major Human Pathogenic Dermatophytic Fungi, Trichophyton rubrum. Front Cell Infection Microbiology. 2015; 5: 57.

[19] Aranda S, Montes-Borrego M, Landa BB. Purple-pigmented violacein producing Duganella spp. Inhabit the Rhizosphere of Wild and Cultiveted Olives in Southern Spain". Microbiology Ecology. 2011; 62: 446-459.