Violacein – Part II: From significance to perspectives

https://doi.org/10.56238/sevened2024.003-037

Luanna de Oliveira e Lima[1], Heivila Monique da Silva Alexandre[2], Carlos Alberto Arcelly Santos Bezerra[3], Larissa Alves da Silva[4], Paulo Bruno Araújo Loureiro[5], Shayenne Eduarda Ramos Vanderley[6], Hueliton Borchardt[7] and Ulrich Vasconcelos[8]

ABSTRACT

Violacein, a bluish-purple pigment formed from the condensation of two tryptophan molecules, acts as a secondary metabolite important for the fitness of its producers in a given ecosystem. These microorganisms are distributed in various environments, including oceans, glaciers, rivers, and soil. Violacein is bioactive and important for the ecological relationships between organisms that coexist with producers. Therefore, the pharmacological potential of the compound can be prospected and the literature provides many examples of how violacein can be used in therapy. This chapter deals with these applications, as well as their obtaining and extraction. The text was prepared by students of the Graduate Program in Natural, Synthetic and Bioactive Products at UFPB, as a final project of the course.

Keywords: Natural bioactives, Bioprospecting, Pharmacological activity.

[1] Pharmaceutical. Master's student in the Graduate Program in Natural Products, Bioactive Synthetics, Federal University of Paraíba, Campus I, 58051-900, João Pessoa-PB
E-mail: luanna@ltf.ufpb.br
ORCID: https://orcid.org/0000-0002-9479-1733
[2] Pharmaceutical. Master's student in the Graduate Program in Natural Products, Bioactive Synthetics, Federal University of Paraíba, Campus I, 58051-900, João Pessoa-PB
E-mail: hmonique@ltf.ufpb.br
ORCID: https://orcid.org/0000-0002-7311-5066
[3] Biotechnologist. Master's student in the Graduate Program in Natural Products, Bioactive Synthetics, Federal University of Paraíba, Campus I, 58051-900, João Pessoa-PB
E-mail: c.alberto7@ltf.ufpb.br
ORCID: https://orcid.org/0009-0005-4562-0304
[4] Pharmaceutical. Master's student in the Graduate Program in Natural Products, Bioactive Synthetics, Federal University of Paraíba, Campus I, 58051-900, João Pessoa-PB
E-mail: larissal21@ltf.ufpb.br
ORCID: https://orcid.org/0000-0001-5073-7033
[5] Pharmacist. Master's student in the Graduate Program in Natural Products, Bioactive Synthetics, Federal University of Paraíba, Campus I, 58051-900, João Pessoa-PB
E-mail: paulobrunoaloureiro@gmail.com
ORCID: https://orcid.org/0009-0001-4349-0236
[6] Biotechnologist. Master's student in the Graduate Program in Natural Products, Bioactive Synthetics, Federal University of Paraíba, Campus I, 58051-900, João Pessoa-PB
E-mail: shayenne.erv@gmail.com
ORCID: https://orcid.org/0000-0001-8459-2717
[7] Graduating in Biotechnology. Universidade Federal da Paraíba, Centro de Biotecnologia, Via Ipê Amarelo, s/n, sala 8, Campus I, 58051-900, João Pessoa-PB.
E-mail: hb@academico.ufpb.br
ORCID: https://orcid.org/0000-0002-9137-9313
[8] Dr. in Chemical and Biochemical Process Engineering. Universidade Federal da Paraíba, Centro de Biotecnologia, Via Ipê Amarelo, s/n, sala 8, Campus I, 58051-900, João Pessoa-PB.
E-mail: u.vasconcelos@cbiotec.ufpb.br
ORCID: https://orcid.org/0000-0001-8289-2230

INTRODUCTION

ROLE OF PIGMENTS FOR MICROORGANISMS

Microbial pigments are secondary metabolites produced for different purposes, among which protection is the most studied target. Protection can be understood individually as it occurs in exposure to ultraviolet radiation and oxidative stress, as well as in a collective way for organisms that coexist. However, pigments take on several other functions, for example, light capture, electron acceptor, and catalyst (Rana et al., 2021; Sutthiwong et al., 2014).

Photoprotection against cell damage is the function guaranteed by the production of reactive oxygen species (ROS). This can also play a vital role in protecting bacterial cells from predation (Rao et al., 2017; Sajjad et al., 2020).

Pigments also play a role in cell differentiation and regulation during cell cycles (Azman et al., 2018; Venil et al., 2020). This is because some of these molecules are involved in cell density-dependent mechanisms. The function of autoinducers attributed to these compounds may also be associated with the production of biofilm, thus contributing to the virulence of these microorganisms (Rao et al., 2017; Sajjad et al., 2020).

SIGNIFICANCE OF VIOLACEIN FOR ITS PRODUCERS

The production of violacein is not specific to a bacterial species like some other pigments. At least six genera have been described, with emphasis on two species, Chromobacterium violaceum *and* Janthinobacterium lividum. The production of violacein by these bacteria is the target of different hypotheses about its biological and ecological purpose (Cauz *et. al*, 2019).

The diversity of violacein producers found in different environments, such as oceans, rivers, lakes, and soil, suggests that compost may play a crucial role in the events associated with the adaptation and maintenance of these microorganisms. In this context, the most important hypothesis is that violacein acts as a defense mechanism, since most of the producing species live in sessile communities, reinforced by the reports of violacein-producing bacteria having a greater capacity to resist antibiotics. Thus, by expressing the pigment, the cell is given a competitive advantage (Lichstein; van de Sand, 1945; Nakamura et al., 2002; Matz et al., 2008).

In addition, violacein exhibits antibacterial properties, particularly against Gram-positive bacteria (*Staphylococcus aureus*) (Nakamura *et al*., 2002), as well as exhibiting antiparasitic activity (*Caenorhabditis elegans* – Nematoda). The demonstration of activity against prokaryotes and eukaryotes suggests that violacein may play a role in the complex interaction between different organisms in a given ecosystem (Matz et al., 2008), for example, violacein promotes the formation of biofilms in frogs and salamanders, which have the advantage of this association, antifungal

protection, contributing to the increase in the life expectancy of these amphibians (Brucker et al., 2008).

In addition, it is known that the production of this pigment only occurs under aerobic conditions (Durán and Faljoni-Alario, 1980) and seems to be associated with a series of adaptive strategies that benefit bacteria in different environmental contexts. However, a full understanding of the biological purpose of violacein requires further investigation, in terms of mechanisms of action and impact on ecological interactions (Melo, 2000).

VIOLACEIN EXTRACTION

The literature reports a crude violacein yield between 1 and 12.6 g (pure, from 0.750 to 1.6 mg) Different methods can be used in the synthesis and extraction of violacein, and there is no model protocol that best represents it, as it depends on the species used, as well as the strain, however polar and nonpolar organic solvents, in which ethanol brings some advantages in the use (Sasidharan et al., 2015).

Rettori and Durán (1998) obtained 1 mg of *C. violaceum* pigment CCT 3496 in medium with glucose, peptone and yeast extract, from inoculation at 30°C for 24 hours. Cotton flakes were inserted in the middle to retain the pigment, which was later extracted with ethanol and purified with chloroform and methanol, obtaining the crystals by centrifugation.

Kanelli et al. (2018) used nutrient broth and observed the optimal condition of the J. *lividum crop* at 150 rpm, 25°C and pH 7.0. The pigment was extracted with methanol and ethyl acetate, optimizing the process by adding antibiotics to the growth medium. Sasidharan et al. (2015) explored the production capacity of violacein by the strain *Chromobacterium* sp. NIIST MTCC5522, using Luria Bertani (LB) broth, under agitation at 150 rpm at 33°C. Subsequently, ethanol and methanol were added, obtaining 1.6 g/L of violacein.

Ahmed et al. (2012) investigated the growth of C. violaceum under agitation at 200 rpm at 30°C for 24 h, using unconventional substrates (sugarcane bagasse, pineapple solid residue, brown sugar and molasses) and obtained 0.82 g/L of crude *violacein.* Removing chloride from the growth medium allowed two strains, *Janthinobacterium sp. p102 and* Massilia *sp. p117*, to produce a similar amount between 5 and 7 days of incubation (Kuzyk et al., 2021). In addition, aiming at the optimization of violacein production by the strain *C. violaceum* MTCC 2656, Subramaniam; Ravi and Sivasubramaniam (2014) varied concentrations of LB broth, with the best result in the condition at 0.625% at 25°C (0.48 mg/mL).

The use of yeasts as a substitute for traditionally used strains allows for the acceleration and reduction of costs in the process (Kholany et al., 2020), as well as reducing the exposure of the

handler to violacein-producing pathogens, based on reports of opportunistic infections associated with the representative species producing the molecule (Yang and Li, 2011).

Yarrowia lipolytica (Ascomicota) is a yeast of biotechnological importance, being reported to produce between 15.4 and 15.76 mg/L of violacein (Nemer et al., 2023), employing simplified media added surfactants, as well as simpler extraction techniques. Although there are mass losses after the purification phase of crude violacein, a fact also observed in bacteria, the potential of yeast, combined with the safety of its handling, make its use much more attractive.

ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF VIOLACEIN

Inspired by the ecological role of bioactive molecules, the potential of microbial pigments is widely explored, given the issue of the increase in antibiotic-resistant pathogens, as well as the limited number of drugs that can combat them (Sajjad, et al., 2020). Different approaches to violacein have shown a wide range of biological properties, including antimicrobial activity (Durán et al 2021; Ahmed et al 2021), which in this text will be understood as activity against planktonic cells, while antibiofilm activity for those of sessile life.

From a study with liposomes formulated with bacterial phospholipids, it was observed that the mechanism of action of violacein is involved in the interference and rupture of the cytoplasmic membrane (Cauz et al., 2019). Brooks et al. (2023) identified inhibitory concentrations between 8 and 4 mg/mL of violacein extract against oral pathogens associated with halitosis, in particular *Porphyromonas gingivalis, Fusobacterium nucleatum, and Solanobacterium moorei.*

Recently, an isolate of a new species of *Janthinobacterium* sp., which produces violacein, proved antimicrobial activity with important pathogens, namely: *Enterococcus faecalis, Yersinia pseudotuberculosis*, Staphylococcus aureus*, Bacillus cereus* and *Candida albicans,* demonstrating the broad spectrum that the pigment expresses (Inan Bektas et al., 2023). In addition, there are also a number of studies carried out with fungi. In a recent review, several fungal genera of clinical and environmental importance were listed, and an important role of violacein in the ecology of fungi was recognized (Durán et al., 2022).

The knowledge of the mechanism by which violacein acts in fungi is still uncertain, however some studies have related the general mechanisms of known antifungals, such as azoles, that is, at the membrane level, acting on the synthesis of ergosterol. Violacein also exhibits a similar action to echinocandins, because it causes damage to the cell wall (Ghannoum and Rice, 1999; Bhattacharya et al., 2020; Howard et al., 2020). It is believed that the pigment promotes this response because it acts to induce the generation of ROS, and can also affect the development of hyphae and the formation of biofilm (Duran et al., 2022).

In vitro *assays* report that the antifungal concentration is higher than that achieved against bacteria, 2 g/mL. Violacein activity has been described against important fungal species such as *Aspergillus niger* and *Candida albicans* (Dike-Ndudim; Ugenyi; Ndubueze, 2021), as well as *Cryptococcus gastricus, Trichophyton rubrum, Fusarium oxysporum, Rhizoctonia solani, Aspergillus flavus, Penicillium expansum, and Trichophyton rubrum* (Sasidharan et al., 2015).

In terms of activity against sessile microorganisms, it was observed that violacein may exhibit antibiofilm activity, either in inhibiting the formation or in the eradication of mature biofilms. The spectrum of organisms was less explored, however 20 and 160 µg/mL of violacein, respectively, eradicated the biofilm at 3 and 2:30 h of incubation, against *Staphylococcus epidermidis* (Dodou et al., 2020).

ANTITUMOR ACTIVITY OF VIOLACEIN

In the context of its antitumor activity, the literature documents extensive research employing violacein in experimental in *vitro* models of cancer. As evidenced by Dahlem et al. (2022), violacein demonstrated cytotoxic effect after 48 hours of treatment, with IC50 values of 0.393 µM for SK-MEL-5 cells and 9.864 µM for HepG2 cells, using the MTT method. In addition, in the same study, the compound in question also exhibited cytotoxicity in a 3D tumor spheroid model in HCT116 cells, having demonstrated the ability to inhibit spheroid growth and promote the displacement of external cells relative to the nucleus. The action triggered by violacein on tumor cell death was further associated with caspase 3 activation and ATP release.

De Souza Oliveira et al. (2022) demonstrated that treatment with violacein for 24 hours resulted in a reduction in the viability of HT29 and HCT116 cells, with the HT29 strain being more sensitive to the compound (0.6 µM IC50) compared to the HCT116 strain (1.2 µM IC50). In addition, the study revealed that violacein works by reducing the expression of tyrosine kinase AXL and Epidermal Growth Factor Receptor (EGFR) receptors in HT29 cells, and by inhibiting the kinases responsible for cell survival and proliferation (ERK, AKT and PKCδ). In addition, there was a decrease in the expression of mesenchymal markers, such as N-cadherin and Snail, and of the β-catenin protein, while the expression of epithelial markers, such as E-cadherin and ZO-1, was increased. These results suggest that violacein overcomes three hallmarks of cancer: growth factor receptor-dependent signaling, proliferation, and Epithelial-Mesenchymal Transition.

A study conducted by Neroni et al. (2022), investigated the antiproliferative activity of violacein in bladder cancer cells, considered the most common type of cancer in urology. It was observed that after 24 hours of exposure to different concentrations of violacein, the IC50 for the T24 strain was 0.1135±0.05 µM, while for the 5637 strain (human bladder urothelial carcinoma) it was 0.1129±0.1 µM. The 5637 strain was shown to be more sensitive to violacein, since after treatment

with 1 µM of the compound for 24 hours, there was a 2% reduction in viability for T24 and a 10% reduction for 5637 (*p<0.0001), compared to untreated cells. The results of this study suggested that violacein may have different modes of action in these cell lines, possibly due to molecular differences between them. However, further investigations are needed to gain a more complete understanding of the violacein-induced cell death mechanism in bladder cancer cells, with particular attention to the divergences observed between the two lineages.*

Berti et al. (2020) developed a system of Active Surface Ionic Liquids (SAILs) that enables the solubility of violacein in aqueous medium. Through screening, the compound [C_{16}Him]-S was selected at a concentration of 1.5×10^3 % (w/v), as it presented a combination of low cytotoxicity, 71.5% cell viability and an effective interaction with 95.2% of violacein maintained in micellar solution for at least 48 hours. The [SLN-Viol-([C $_{16}$ Him]-S)-FA] complex was employed in the development of an efficient hybrid solid lipid (SLN) nanoparticle transporter, resulting in a five-fold higher incorporation of violacein nanoparticles into HCT116 and HeLa cell cultures, demonstrating a high level of folate receptor affinity.

Bromberg et al. (2010) tested violacein in in *vitro* and *in vivo* models. The cytotoxic effect was observed in Ehrlich cells (5 µM IC50), considered the most sensitive when compared to normal human peripheral blood lymphocytes. The researchers showed that the cytotoxic effects were due to the increase in reactive oxygen species (ROS) and the decrease in glutathione levels (GHS). In addition, apoptosis was induced by activation of caspases 2, 3 and 9 after treatment for 72 hours. In Ehrlich's ascitic tumor models in mice, violacein significantly inhibited tumor growth and increased the survival rate of the animals. Tests did not indicate hematotoxicity, nephrotoxicity, and hepatotoxicity.

According to Kim et al. (2021), violacein inhibited the proliferation of hepatocellular carcinoma (HCC) in Huh7 strains, with IC50 values of 7.97, 6.71, and 6.10 µM at 24, 48, and 72 h, respectively, and in Hep3B strains, with IC 50 values of 8.01, 8.41, and 8.23 µM in the same time interval. This effect was attributed to cell cycle disruption, resulting in a significant increase in the cell population in the sub-G1 phase and a decrease in the cell population in the G0/G1, S, and G2/M phases, compared to the untreated control group, indicative of apoptosis. This violacein-induced effect was associated with nuclear condensation, loss of mitochondrial membrane potential (MMP), increased ROS generation, activation of caspase-9, caspase-3, and PARP, upregulation of p53 and p21, and downregulation of AKT and ERK1/2 signaling pathways. In addition, violacein significantly suppressed the proliferation and formation of tumor stem cell-like spheres of hepatocellular carcinoma by reducing the expression of HCC cellular potentiality markers, including CD133, Sox2, Oct4, and Nanog, and by inhibiting STAT3/AKT/ERK signaling pathways. In

conclusion, these results suggest that violacein has chemotherapeutic potential to effectively suppress HCC, targeting both HCC cell proliferation and potentiality.

ANTI-INFLAMMATORY ACTIVITY OF VIOLACEIN

The versatility of violacein includes antitumor, leishmanicidal, trypanocide, antifungal, antiviral, antibacterial, antiprotozoal, and antinematode effects (Abdelghani et al., 2021; Justo and Durán, 2017), and in addition it also exhibits antioxidant and anti-inflammatory properties, which manifest themselves through direct action on pathways related to the inflammatory response, including the modulation of the immune system (Ballestriero et al., 2014; Durán et al; 2016; Durán et al., 2021).

To date, research on the modulation of the inflammatory response by this pigment has revealed antipyretic, analgesic, and immunomodulatory activities in acute and chronic inflammatory conditions (Choi et al., 2021; Park et al., 2021). Antonisamy and Ignacimuthu (2010) investigated the anti-inflammatory potential of violacein by exploring the clinical pillars of inflammation, which include flushing, pain, warmth, and swelling. The study was conducted on *Wistar* rats and mice, analyzing the analgesic effect through acetic acid-induced contortions, formalin-induced paw licking, and hot plate tests. The research also addressed the immunomodulatory effect of violacein by examining ovalbumin-induced active paw anaphylaxis and sheep red blood cells (SRBC) delayed hypersensitivity tests. In addition, the antipyretic activity was evaluated by yeast-induced hyperpyrexia in rats. To compare the anti-oedema effect, violacein was compared with indomethacin.

The results revealed that violacein inhibited 42.9% of ovalbumin-induced edema, as well as effectively decreased edema caused by sheep red blood cells. In addition, a significant analgesic activity was observed in the contortion tests triggered by acetic acid, in the paw licking response provoked by formalin and in the hot plate tests. Treatment with violacein has demonstrated a significant and dose-dependent reduction in pyrexia in rats (Antonisamy and Ignacimuthu, 2010).

Violacein, in the study by Antonisamy et al. (2014), revealed a remarkable gastroprotective effect against indomethacin-induced lesions in Wistar rats. The activity of myeloperoxidase (MPO), an enzyme important in the inflammatory response, was significantly reduced in the group treated with violacein with a superior ability to reduce MPO levels compared to omeprazole. An increase in the activity of the constitutive nitric oxide synthase (cNOS) enzyme in the gastrointestinal mucosa was also observed with the administration of violacein. On the other hand, the pigment in conjunction with omeprazole significantly restored cyclooxygenase 1 (COX-1)-mediated prostaglandin E2 (PGE2) levels, promoting ulcer healing and repair.

In addition, violacein reduced tumor necrosis factor-α, interleukin-1β, and interleukin-6, as well as increased interleukin-4 and 10, along with growth factors (vascular, endothelial, epidermal,

and hepatocyte), demonstrating a comprehensive therapeutic potential (Antonisamy et al., 2014). These results indicated for the first time in the literature a mechanism by which violacein can reduce inflammation, but also promote an environment conducive to healing, in gastrointestinal ulcers.

Verinaud et al. (2015) observed the broad anti-inflammatory spectrum of violacein, revealing its modulatory potential in different models of inflammation. In acute lipopolysaccharide (LPS)-mediated inflammation, for example, pigment not only preserved the homeostasis of dendritic cells (CD80/86) and T and B lymphocytes, but also exerted a significant impact on the inflammatory response. There was a remarkable reduction in neutrophil migration, indicating an effective modulation of the immune response. In addition, a decrease in the levels of the inflammatory cytokine IL-6 and the chemokine C-X-C ligand 1 (CXCL1) was observed in the mice treated with violacein, while the levels of IL-10 increased substantially. These alterations indicate a violacein-induced anti-inflammatory profile, evidencing its potential to create an environment conducive to immune regulation during acute inflammation.

In *vitro results*, an induction in the production of this pro-inflammatory cytokine was observed. Venegas et al. (2019) observed the effect of violacein on different immune cell lines, namely THP-1, MonoMac 6, ANA-1, Raw 264.7, HEK-293, as well as on human peripheral blood mononuclear cells (PBMCs). Stimulation of TNF-α production in murine macrophages (ANA-1 and Raw 264.7) has been reported, and secretion of IL-6 and IL-1β has been detected in PBMCs. In addition, activation of the inflammatory molecular mechanism after treatment with violacein was observed in Raw 264.7 cells. The treatment caused the activation of pro-inflammatory mediators such as TNF-α, *Immune Responsive Gene 1* (IRG1), chemokines C-C ligand 2 (CCL2), and C-X-C ligand 2 (CXCL2). In addition, it has been reported that in toll-like receptor (TLR)-transfected HEK-293 cells, violacein activated the human receptor TLR8 (hTLR8) signaling pathway and that in PBMCs, this immunomodulatory effect can be suppressed by the hTLR8-specific antagonist.

Based on these reports, violacein is recognized as a promising candidate in comprehensive therapeutic interventions, offering potential not only in reducing inflammation, but also in promoting healing and modulating the immune response. In addition, violacein's property of activating inflammatory mechanisms can be strategically exploited in the treatment of diseases that require greater activation of the immune system, such as some parasitic infections, viruses, and cancer (Doganci et al., 2022; Lopes et al., 2009; Platt et al., 2014).

REFERENCES

1. ABDELGHANI, Z.; HOURANI, N.; ZAIDAN, Z.; DBAIBO, G.; MRAD, M.; HAGE-SLEIMAN, R. Therapeutic applications and biological activities of bacterial bioactive extracts. Arch Microb. v. 203, n. 8, p. 4755–4776, 2021.

2. AHMED, A.; Ahmad, A.; Li, R.; Al-Ansi, W.; Fatima, M.; Mushtaq, B.S.; Basharat, S.; Li, Y.; Bai, Z. Recent advances in synthetic, industrial and biological applications of violacein and its heterologous production. J Microbiol Biotechnol. v. 31, n. 11, p. 1465, 2021.

3. ANTONISAMY, P.; IGNACIMUTHU, S. Immunomodulatory, analgesic and antipyretic effects of violacein isolated from Chromobacterium violaceum. Phytomedicine. v. 17, n. 3–4, p. 300–304, 2010.

4. ANTONISAMY, P.; KANNAN, P.; ARAVINTHAN, A.; DURAIPANDIYAN, V.; VALAN ARASU, M.; IGNACIMUTHU, S.; ABDULLAH AL-DHABI, N.; KIM, J. H. Gastroprotective activity of violacein isolated from Chromobacterium violaceum on indomethacin-induced gastric lesions in rats: investigation of potential mechanisms of action. Sci World J. v. 2014, 2014. doi: 10.1155/2014/616432.

5. AZMAN, A.S.; MAWANG, C.I.; ABUBAKAR, S. Bacterial pigments: The bioactivities and as an alternative for therapeutic applications. Nat Product Commun. v. 13, n. 12, 2018. https://doi.org/10.1177/1934578x1801301240.

6. BALLESTRIERO, F.; DAIM, M.; PENESYAN, A.; NAPPI, J.; SCHLEHECK, D.; BAZZICALUPO, P.; SCHIAVI, E. DI; EGAN, S. Antinematode activity of violacein and the role of the insulin/IGF-1 pathway in controlling violacein sensitivity in Caenorhabditis elegans. Plos One, v. 9, n. 10, p. e109201, 2014. doi: 10.1371/journal.pone.0109201.

7. BERTI, I.R.; RODENAK-KLADNIEW, B.; ONAINDIA, C.; ADAM, C.G.; ISLAN, G.A.; DURÁN, N.; CASTRO, G.R. Assessment of in vitro cytotoxicity of imidazole ionic liquids and inclusion in targeted drug carriers containing violacein. RSC Adv. v. 10, n. 49, p. 29336-29346, 2020.

8. BHATTACHARYA, S.; SAE-TIA, S.; FRIES, B.C. Candidiasis and mechanisms of antifungal resistance. Antibiotics. v. 9, n. 6, p. 312, 2020. doi: 10.3390/antibiotics9060312.

9. BROMBERG, N.; DREYFUSS, J.L.; REGATIERI, C.V.; PALLADINO, M.V.; DURAN, N.; NADER, H.B.; JUSTO, G.Z. Growth inhibition and pro-apoptotic activity of violacein in Ehrlich ascites tumor. Chem Biol Interact. v. 186, n. 1, p. 43-52, 2010.

10. BROOKS, J.S.; LOURENÇO, T.G.B.; RURR, J.S.C.; COLOMBO, A.P.V. Antimicrobial activity of violacein against oral bacteria associated with halitosis: an in vitro study. Rev Cient CRO-RJ. v. 8, n. 1, p. 14-20, 2023.

11. BRUCKER, R.M.; HARRIS, R.N.; SCHWANTES, C.R.; GALLAHER, T.N.; FLAHERTY, D.C.; LAM, B.A.; MINBIOLE, K.P.C. Amphibian chemical defense: Antifungal metabolites of the microsymbiont Janthinobacterium lividum on the salamander Plethodon cinereus. J Chem Ecol. v. 34, p. 1422-1429, 2008.

12. CAUZ, A.C.G.; CARRETERO, G.P.B.; SARAIVA, G.K.V.; PARK, P.; MORTARA, L.; CUCCOVIA, I.M.; BROCCHI, M.; GUEIROS-FILHO, F.J. Violacein targets the cytoplasmic membrane of bacteria. ASC Infect Dis. v. 5, n. 4, p. 539-549, 2019.

13. CHO, S. Y.; LIM, S.; YOON, K. HYE; LEE, J. I.; MITCHELL, R. J. Biotechnological activities and applications of bacterial pigments violacein and prodigiosin. J Biol Eng. v. 15, n. 1, p. 1–16, 2021.

14. DAHLEM, C.; CHANDA, S.; HEMMER, J.; SCHYMIK, H.S.; KOHLSTEDT, M.; WITTMANN, C.; KIEMER, A.K. Characterization of anti-cancer activities of violacein: actions on tumor cells and the tumor microenvironment. Front Oncol. v. 12, p. 872223, 2022. doi: 10.3389/fonc.2022.872223.

15. DE SOUZA OLIVEIRA, P.F.; FARIA, A.V.; CLERICI, S.P.; AKAGI, E.M.; CARVALHO, H.F.; JUSTO, G.Z.; FERREIRA-HALDER, C.V. Violacein negatively modulates the colorectal cancer survival and epithelial–mesenchymal transition. J Cell Biochem. v. 123, n. 7, p. 1247-1258, 2022.

16. DIKE-NDUDIM, J.N.; UGENYI, L.C.; NDUBUEZE, C.W. Assessment of antifungal potentials of violacein extract from Chromobacterium violaceum isolated from domestic and recreational water sources in Owerri, Imo State, Nigeria. World J Adv Res Rev. v. 10, n. 3, p. 168-172, 2021.

17. DODOU, H.V.; BATISTA, A.H.M.; MEDEIROS, S.C; SALES, G.W.P.; RODRIGUES, M.L.; PEREIRA, P.I.O.; NOGUEIRA, P.C.N.; SILVEIRA, E.R.; GRANGEIRO, T.B.; NOGUEIRA, A.P. Violacein antimicrobial activity on Staphylococcus epidermidis biofilm. Nat Product Res. v. 34, n. 23, p. 3414-3417, 2020.

18. DOGANCI, M. A.; AY SAL, F.; GULER, H. I.; KATI, H.; CEYLAN, E.; BELDUZ, A. O.; BOZDAL, G.; YAYLI, N.; CANAKCI, S. Investigation of potential inhibitor properties of violacein against HIV-1 RT and CoV-2 Spike RBD:ACE-2. World J Microb Biotechnol. v. 38, n. 9, p. 1–14, 2022.

19. DURÁN, N.; CASTRO, G.R.; PORTELA, R.W.D.; FÁVARO, W.J.; DURÁN, M.; TASIC, L.; NAKAZATO, G. Violacein and its antifungal activity: comments and potentialities. Lett Appl Microbiol. v. 75, n. 4, p. 796-803, 2022.

20. DURÁN, N.; FALJONI-ALARIO, A. 1980. Bacterial chemistry-I: studies of a potencial phototherpaeutic substance from Chromobacterium violaceum. An. Acad Bras Ciênc. v. 52, p. 297-302, 1980.

21. DURÁN, N.; JUSTO, G. Z.; DURÁN, M.; BROCCHI, M.; CORDI, L.; TASIC, L.; CASTRO, G. R.; NAKAZATO, G. Advances in Chromobacterium violaceum and properties of violacein-Its main secondary metabolite: A review. Biotechnol Adv. v. 34, n. 5, p. 1030–1045, 2016.

22. DURÁN, N.; NAKAZATO, G.; DURÁN, M.; BERTI, I. R.; CASTRO, G. R.; STANISIC, D.; BROCCHI, M.; FÁVARO, W. J.; FERREIRA-HALDER, C. V.; JUSTO, G. Z.; TASIC, L. Multi-target drug with potential applications: violacein in the spotlight. World J Microb Biotechnol. v. 37, n. 9, p. 1–20, 2021.

23. GHANNOUM, M.A.; RICE, L.B. Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev. v. 12, n. 4, p. 501-517, 1999.

24. HOWARD, K.C.; DENNIS, E.K.; WATT, D.S.; GARNEAU-TSODIKOVA, S. A comprehensive overview of the medicinal chemistry of antifungal drugs: perspectives and promise. Chem Soc Rev. v. 49, n. 8, p. 2426-2480, 2020.

25. INAN BEKTAS, K.; NALCAOGLU, A.; KATI, H.; CEYLAN, E.; NALCACIOGLU, R.; BELDUZ, A.O.; CANAKCI, S. Janthinobacterium kumbetense sp. nov., a violacein-producing bacterium isolated from spring water in Turkey, and investigation of antimicrobial activity of violacein. FEMS Microbiol Lett. v. 370, p. 119, 2023. doi: 10.1093/femsle/fnac119.

26. JUSTO, G. Z.; DURÁN, N. Action and function of Chromobacterium violaceum in health and disease: Violacein as a promising metabolite to counteract gastroenterological diseases. Best Pract Res Clin Gastroenterol. v. 31, n. 6, p. 649–656, 2017.

27. KANELLI, M.; MANDIC, M.; KALAKONA, M.; VASILAKOS, S.; KEKOS, D.; NIKODINOVIC-RUNIC, J.; TOPAKAS, E. Microbial production of violacein and process optimization for dyeing polyamide fabrics with acquired antimicrobial properties. Front Microbiol. v. 9, p. 1495, 2018. doi: 10.3389/fmicb.2018.01495.

28. KHOLANY, M.; TRÉBULLE, P.; MARTINS, M.; VENTURA, S.P.M.; NICAUD, J-M.; COUTINHO, J.A.P. Extraction and purification of violacein from Yarrowia lipolytica cells using aqueous solutions of surfactants. J Chem Technol Biotechnol. v. 95, n. 4, p. 1126-1134, 2020.

29. KIM, Y.J., YUK, N., SHIN, H.J., JUNG, H.J. The natural pigment violacein potentially suppresses the proliferation and stemness of hepatocellular carcinoma cells in vitro. Int J Molec Sci. v. 22, n. 19, p. 10731, 2021.

30. KUZYK, S.; PRITCHARD, A.O.; PLOUFFE, J.; SORENSEN, J.L.; YURKOV, V. Psychrotrophic violacein-producing bacteria isolated from Lake Winnipeg, Canada. J Great Lakes Res. v. 47, n. 3, p. 715-724, 2021.,

31. LICHSTEIN, H.C.; VAN DE SAND, V.F. Violacein, an antibiotic pigment produced by Chromobacterium violaceum. J Infect Dis. v. 76, n. 1, p. 47-51, 1945.

32. LOPES, S. C. P.; BLANCO, Y. C.; JUSTO, G. Z.; NOGUEIRA, P. A.; RODRIGUES, F. L. S.; GOELNITZ, U.; WUNDERLICH, G.; FACCHINI, G.; BROCCHI, M.; DURAN, N.; COSTA, F. T. M. Violacein extracted from Chromobacterium violaceum inhibits plasmodium growth in vitro and in vivo. Antimicrob Agent Chemother. v. 53, n. 5, p. 2149, 2009.

33. MATZ, C.; WEBB, J.S.; SCHUPP, P.S.; PHANG, S.Y.; PENESYAN, A.; EGAN, S.; STEINBERG, P.; KJELLEBERG, S. Marine biofilm bacteria evade eukaryotic predation by targeted chemical defense. PloS One, v. 3, n. 7, p. e2744, 2008. doi: 10.1371/journal.pone.0002744.

34. MELO, P.S.; MARIA, S.S.; VIDAL, B.C.; HAUN, M.; DURÁN, N. Violacein cytotoxicity and induction of apoptosis in V79 cells. In Vitro Cell Dev Biol Anim. v. 36, n. 8, p. 539-543, 2000.

35. NAKAMURA, Y.; SAWADA, T.; MORITA, Y.; TAMIYA, E. Isolation of a psychrotrophic bacterium from the organic residue of a water tank keeping rainbow trout and antibacterial effect of violet pigment produced from the strain. Biochem Eng J. v. 1, p. 79-86, 2002.

36. NEMER, G.; LOUKA, N.; BLANDIN, P.R.; MAROUN, R.G.; VOROBIEV, E.; ROSSIGNOL, T.; NICAUD, J-M.; GUÉNIN, E.; KOUBAA, M. Purification of natural pigments violacein and deoxyviolacein produced by fermentation using Yarrowia lipolytica. Molecules. v. 28, n. 11, p. 4292. doi: 10.3390/molecules28114292.

37. NERONI, B.; ZINGAROPOLI, M.A.; RADOCCHIA, G.; CIARDI, M.R.; MOSCA, L.; PANTANELLA, F.; SCHIPPA, S. Evaluation of the anti proliferative activity of violacein, a

natural pigment of bacterial origin, in urinary bladder cancer cell lines. Oncol Lett. v. 23, n. 4, p. 1-9, 2022.

38. PARK, H. A.; PARK, S. A.; YANG, Y. H.; CHOI, K. Y. Microbial synthesis of violacein pigment and its potential applications. Crit Rev Biotechnol. v. 41, n. 6, p. 879–901, 2021.

39. PLATT, D.; AMARA, S.; MEHTA, T.; VERCUYSSEE, K.; MYLES, E. L.; JOHNSON, T.; TIRIVEEDHI, V. Violacein inhibits matrix metalloproteinase mediated CXCR4 expression: Potential anti-tumor effect in cancer invasion and metastasis. Biochem Biophys Res Comm. v. 455, n. 1–2, p. 107–112, 2014.

40. RANA, B; BHATTACHARYYA, M; PATNI, B; ARYA, M; JOSHI, G. K. The realm of microbial pigments in the food color market. Front Sustain Food Syst. v. 5, n. 603892, 2021. doi: 10.3389/fsufs.2021.603892.

41. RAO, N.; PRABHU, M.; XIAO, M.; LI, W. J. Fungal and bacterial pigments: secondary metabolites with wide applications. Front Microbiol. v. 8, n. 1113, 2017. doi: 10.3389/fmicb.2017.

42. RETTORI, D.; DURÁN, N. Production, extraction and purificationof violacein: an antibiotic pigment producedby Chromobacterium violaceum. World J Microbiol Biotechnol. v. 14, p. 685–688, 1998.

43. SAJJAD, W.; DIN, G.; RAFIQ, M.; IQBAL, A.; KHAN, S.; ZADA, S.; ALI, B.; KANG, S. Pigment production by cold-adapted bacteria and fungi: colorful tale of cryosphere with wide range applications. Extremophiles. v. 24, n. 4, 2020. doi 10.1007/s00792-020-01180-2.

44. SASIDHARAN, A.; SASIDHARAN, N.K.; AMMA, D.B.N.S.; VASU, R.K.; NATARAJA, A.V.; BHASKARAN, K. Antifungal activity of violacein purified from a novel strain of Chromobacterium sp. NIIST (MTCC 5522). J Microbiol. v. 53, p. 694-701, 2015.

45. SUBRAMANIAM, S.; RAVI, V.; SIVASUBRAMANIAN, A. Synergistic antimicrobial profiling of violacein with commercial antibiotics against pathogenic micro-organisms. Pharmac Biol. v. 52, n. 1, p. 86-90, 2014.

46. SUTTHIWONG, N.; FOUILLAUD, M; VALLA, A; CARO, Y; DUFOSSÉ, L. Bacteria belonging to the extremely versatile genus Arthrobacter as a novel source of natural pigments with extended hue range. Food Res Int. v. 65, 2014. doi: 10.1016/j.foodres.2014.06.024.

47. VENEGAS, F. A.; KÖLLISCH, G.; MARK, K.; DIEDERICH, W. E.; KAUFMANN, A.; BAUER, S.; CHAVARRÍA, M.; ARAYA, J. J.; GARCÍA-PIÑERES, A. J. The bacterial product violacein exerts an immunostimulatory effect via TLR8. Sci Rep. v. 9, n. 1, p. 1–17, 2019.

48. VENIL, C.K.; VELMURUGAN, P.; DUFOSSÉ, L.; DEVI, P.R.; RAVI, A.V. Fungal pigments: potential coloring compounds for wide ranging applications in textile dyeing. J Fungi. v. 6, n. 68, 2020. doi: 10.3390/jof6020068.

49. VERINAUD, L.; LOPES, S.C.P.; PRADO, I.C.N.; ZANUCOLI, F.; COSTA, T.A.; DI GANGI, R.; ISSAYAMA, L.K.; CARVALHO, A.C.; BONFANTI, A.P.; NIEDERAUER, G.F.; DURÁN, N.; COSTA, F.T.M.; OLIVEIRA, A.L.R.; HÖFLING, M.A.C.; MACHADO, D.R.S.; THOMÉ, R. Violacein treatment modulates acute and chronic inflammation through the suppression of cytokine production and induction of regulatory T cells. Plos One, v. 10, n. 5, p. e0125409, 2015. doi: 10.1371/journal.pone.0125409.

50. YANG, C-H.; LI, Y-H. Chromobacterium violaceum infection: a clinical review of an important but neglected infection. J Chin Med Assoc. v. 74, n. 10, p. 435-441, 2011.