DOI 10.47176/sjmshm.6.1.1

Original Article

SRPH

SJMSHM, 2024; 6(1): 1-11

Investigating the Antioxidant, Cytotoxic and Antimicrobial Effects of a Red Pigment Obtained from Marine Bacterial Strain

Zahra Sabouri[1]*, Parmis Ghorbani[2]

[1,2] Department of Industrial Microbiology, Islamic Azad University, Shiraz, Iran.

***Corresponding Author:**
zsaboori069@gmail.com

Received: 15 October, 2023
Accepted: 22 February, 2024
Published: 25 March, 2024

ABSTRACT

Synthetic pigments have been widely used in various applications since the 1980s. However, the hyperallergenicity or carcinogenicity effects of synthetic dyes have led to the increased research on natural pigments. Among the natural resources, bacterial pigments are a good alternative to synthetic pigments because of their significant properties. Nontoxic nature of pigment produced by a number of microorganisms make them environmentally friendly for utilization in dye, foodstuff, pharmacy, cosmetics and other industrial purposes. Bacterial pigments are promising compounds in the prevention and treatment of various cancers. In the current study, the antioxidant, cytotoxic and antimicrobial effects of a red pigment obtained from a marine bacterial strain were investigated. Optimization of the pigment production by the marine strain was conducted using the one-factor-at-a-time approach. Chemical identification of the pigment was achieved by UV-visible, FTIR and HPLC analyses. The biological activities of the pigment were evaluated by DPPH, MTT and microbroth dilution assays. The strain was identified as Arthrobacter, and its pigment was related to carotenoids. The EC50 antioxidant activity of the pigment was evaluated as 4/5 mg ml1. It showed moderate anticancer effects on an oesophageal cancer cell line, KYSE30, while no inhibition was observed on normal HDF (human dermal fibroblasts) cells. The pigment had no antibacterial effects on the four tested strains. In this study the antitumour activity of a carotenoid-related pigment from Arthrobacter sp. was reported for the first time. Marine environments are interesting sources for the identification of novel bioproducts. The identification of carotenoid pigments from marine bacteria with remarkable antioxidant and anticancer activities would result in better insights into the potential pharmaceutical applications of carotenoids and marine environments.

Keywords: *Bacterial pigments, microorganism, antimicrobial activities, biocolorants*

Introduction

The new found awareness in human safety and environmental conservation has kindled fresh enthusiasm for natural sources of colors. Natural colorants or dyes derived from flora and fauna are believed to be safe because of non-toxic, non-carcinogenic and biodegradable in nature [1]. Traditional sources of colorants include natural products such as flavonoids and anthraquinones produced by plants and animals. For example, carminic acid, a deep red anthraquinone produced by scale insects is now used as a pigment in paints, crimson ink, cosmetics and food colors [2]. As the present trend throughout the world is shifting towards the use of ecofriendly and biodegradable commodities, the demand for natural

[DOI: 10.47176/sjmshm.6.1.1]

Publisher: Scientific Research Publishing House (SRPH), Shirvan, Iran
http://srpub.org
Email: sjmshm@srpub.org

colorants is increasing day by day. Natural pigments are sourced from ores, insects, plants and microbes. Among microbes, bacteria have immense potential to produce diverse bioproducts and one such bioproduct is pigments. The production and application of bacterial pigments as natural colorants has been investigated by various researchers [3–5]. Bacterial pigment production is now one of the emerging fields of research to demonstrate its potential for various industrial applications [4]. Most of the bacterial pigment production is still at the R&D stage. Hence, work on the bacterial pigments should be intensified especially in finding cheap and suitable growth medium which can reduce the cost and increase its applicability for industrial production [5]. There are many studies in the literature on bacterial pigments which focus production and application of specified pigment in each case. A comprehensive material of such studies on bacterial pigments is rare in literature. So, based on accumulated data in the literature and with an intention to encourage researchers and stake holders alike to explore and exploit this overflowing source of bacterial pigments.

History of pigments

Dyeing was known as early as in the Indus Valley period (2600–1900 BC); this knowledge has been substantiated by findings of colored garments of cloth and traces of madder dye in the ruins of the Indus Valley Civilization at Mohenjodaro and Harappa. So, natural dyes, dyestuff and dyeing are as old as textiles themselves. Man has always been interested in colors; the art of dyeing has a long past and many of the dyes go back into prehistory. It was practiced during the Bronze Age in Europe. The earliest written record of the use of natural dyes was found in China dated 2600 BC [6]. Primitive dyeing techniques included sticking plants to fabric or rubbing crushed pigments into cloth. The methods became more sophisticated with time and techniques using natural dyes from crushed fruits, berries and other plants, which were boiled into the fabric and which gave light and water fastness (resistance), were developed. The cochineal dye was used by the people of Aztec and Maya culture period of Central and North America. By the 4th century AD, dyes such as woad, madder, weld, Brazil wood, indigo and a dark reddish-purple were known [7]. Henna was used even before 2500 BC, while saffron is mentioned in the Bible [8]. Use of natural pigments in food is known from Japan in the shosoin text of the Nara period (8th century) which contains references to colored soybean and adzuki-bean cakes, so it appears that colored processed foods had been taken at least by people of some sections. Thus, studies on natural pigments are greatly impulse by their multiple functions [9]. The art of coloring spread widely with the advancement of civilization [10]. Before the advent of synthetic pigments, natural pigments were the only source of color available and were widely used and traded, providing a major source of wealth creation around the globe. It has been used for many purposes such as the coloring of natural fibers (wool, cotton, silk), fur and leather. They were also used to color cosmetic products and to produce inks, watercolors and artist's paints [1]. Since the introduction of synthetic dyes by Perkin in 1856, many convenient and cheap synthetic pigments have appeared, and the use of natural dyes has decreased due to the relatively cheaper synthetic pigments [11]. Over the course of the 20th century, naturally occurring organic pigments have been almost completely displaced by synthetic molecules such as phthalocyanines that range from blue to green, arylides that are yellow to greenish or reddish-yellow and quinacridones ranging from orange to violet [12]. Advances in organic chemistry enabled mass production of these compounds relatively cheaper thereby allowing them to displace natural product pigments, whose procurement is often more challenging. Current applications of synthetic pigments are in the textile industry, leather tanning industry, paper production, food technology, agricultural research, light-harvesting arrays, photo electrochemical cells and in hair colorings.

Biopigments types, classification and properties

Colors are present in the nature. Microorganisms such as fungi, yeasts and bacteria produce a vast variety of biopigments such as carotenoids, melanins, flavins, quinones, monascins, violacein or indigo [13]. According to Delgado-Vargas et al. (2000), natural pigments can be classified as follows based on their structural characteristics:

- Tetrapyrrole derivatives: chlorophylls and heme colors.
- Isoprenoid derivatives: carotenoids and iridoids.
- N-heterocyclic compounds different from tetrapyrroles: pterins, purines, flavins, phenoxazines, phenazines and betalains.
- Benzopyran derivatives (oxygenated heterocyclic substances): anthocyanins and alternative flavonoid pigments.
- Quinones: benzoquinone, naphthoquinone, anthraquinone.
- Melanins.

[DOI: 10.47176/sjmshm.6.1.1]

Tetrapyrrole derivatives have pyrrole rings in linear or cyclic arrays. Pyrrole is a colorless liquid substance, soluble in alcohol, ether, diluted acids and also in most organic chemicals (National Center for Biotechnology Information, 2018a). Chlorophyll structure contains a hydrocarbon tail connected to chlorin, an aromatic ring with tetrapyrrole. The magnesium ion in the center is bound to four pyrrole rings.

Chlorophyll is a green pigment found in most algae, plants and cyanobacteria. Chlorophyll a, d and f are present in cyanobacteria. It is a flammable liquid with antitumor properties, antioxidant activities [14] and its derivatives have been found effective against dengue vectors [15].

In general, carotenoids have eight isoprenoid units whose order is inverted at the molecule center. All carotenoids can be considered as lycopene (C40H56) derivatives by reactions adding or removing hydrogen, inserting oxygen, of cyclization, of double bond or methyl migration, of chain elongation and shortening [16]. Lycopene is a solid red bright pigment, used to add color in food and to stabilize flavor formulation (NCBI, 2018c), with antioxidant, anti-inflammatory, and neuroprotective properties [17]. b-Carotene can be a red, brownish-red or purple-violet crystal or powder, depending on the solvent used for extraction and conditions of crystallization. Carotenoids are soluble in benzene, chloroform, carbon disulfide and moderately soluble in ether, petroleum ether and oils. B Carotene is approved by U.S. Food and Drug Association to be used as a nutrient supplement and a as a source of vitamin A. Direct exposure of caretenoids can cause eye and skin irritation and harmful to aquatic life with long lasting effects (NCBI, 2018d). In flavins, a pteridine and a benzene ring are condensed. Riboflavin, or vitamin B2, is the main molecule of this group, produced by all live cells of microorganisms and plants shortening [18]. It is a solid orange-yellow pigment, soluble in water, sodium chloride solution and dilute alkali solution [19]. Based on the structure, they can be divided into the yellow betaxanthins (480 nm) and red-purple betacyanins (540 nm) and their color is due to the resonating double bonds (NCBI, 2018f). Betalains present antioxidant, antiradical, antimicrobial and neuroprotective properties [17]. Among the flavonoids, the anthocyanins are the most relevant pigments. Chemically, they have 15 carbons with a chrome ring bearing a second aromatic ring B in position 2 (C6-C3-C6) and with sugar molecules bonded at different hydroxylated positions of the primary structure, which gives to them several possibilities of combinations and production of colors. They possess antimicrobial and antioxidant activities and can reduce the risk of cancer and heart disease shortening [15].

Melanins are nitrogenous polymeric compounds with the indole ring as monomer. In general, melanins are a mixture of macromolecules, characterized by molecular irregularity, photoconductivity and insolubility at most used solvents [18]. Allomelanins have been described in spores and fungi shortening.

Types of bacterial pigment

Carotenoids: Carotenoids are one of the main natural pigments found widely in plant and microorganisms. These pigments are lipid soluble, occurs in yellow-orange-red color and belongs to the isoprenoid polyenes [19]. In bacteria, carotenoid may play a role in the modulation of membrane fluidity to survive under low temperature conditions. Carotenoid also provides protection to bacterial cells by absorbing or screening UV radiation [3]. Canthaxanthin is a carotenoid with orange-pink color and was first isolated from Micrococcus roseus [1] while echinenone, an orange carotenoid also obtained from the same bacteria was later identified in 1970 [2]. Besides that, an extremely halophillic bacteria, Haloferax alexandrines and a photosynthetic bacterium, Bradyrhizobium sp. also can produce dark-red canthaxanthin. Astaxanthin, a red or oxygenated carotenoid [13], is one of the most abundant marine pigments and cannot be synthesized by animals. It is a naturally lipid-soluble pigment that is mostly found in marine water bacteria such as Halobacterium sp., Brevundimonas sp. Strain SD212, Paracoccus species, Micrococcus sp. strain PAH83, Altererythrobacter ishigakiensis, and Sphingomicrobium astaxanthinifaciens. However, astaxanthin was also obtained in non-marine bacteria such as Sphingomonas sp. from cold storage. Staphyloxanthin, an orange membrane-bound carotenoid found in Staphylococcus aureus gives gold color to cells and acts as a virulence factor for the bacteria itself. Xanthomonas oryzae pv. Oryzae is a causal agent of a rice plant disease called bacterial leaf blight, and produces yellow membrane bound and brominated aryl-polyene pigments [15]. However, the presence of a unique class of carotenoids known as xanthomonadins has led them to be used as diagnostics. Flexirubin, first isolated in 1974 from an aerobic Gram-negative gliding bacterium, Chitinophaga filiformis (previously known as Flexibacter elegans) is a unique type of bacterial pigment with terminal alkyl substitution consisting of ω- phenyl octaenic acid

[DOI: 10.47176/sjmshm.6.1.1]

chromophore esterified with resorcinol [16]. Flexirubin has a restricted distribution among bacteria and has been used as chemotaxonomic markers for the Bacteroidetes phylum, previously called the Cytophaga-Flavobacterium-Bacteroides group [17].

Prodigiosin: Prodigiosin is a linear tripyrrole and a red pigment secondary metabolite. It was first extracted from Serratia marcescens [18]. It is sensitive to light and insoluble in water, but soluble in alcohol and ether such as methanol, chloroform and acetonitrile [19]. In nature, prodigiosin is produced only in the later stages of bacterial growth [20]. Prodigiosin is produced in Gram negative bacteria such as Pseudomonas magnesiorubra, Serratia rubidaea, Alteromonas rubra, Vibrio gazogenes, Zooshikella rubidus, and Hahella chejuensis. Some actinobacteria producing prodigiosin include Streptomyces griseoviridi, Streptomyces sp. JS520, and Streptoverticillium rubrireticuli. Prodigiosin has no defined role in the physiology of producing strains [21] but have been reported to have different in vitro bioactivities.

Melanin: Melanin are dark colored natural pigments, formed due to polymerized phenolic and/or indolic compounds [11]. Melanin can be classified into three classes based on color and structure (i) eumelanins (black brown), (ii) pheomelanins (red or yellow) and (iii) allomelanins (dark brown to black). This pigment is found widely dispersed in animals, plants and microorganisms. Research on melanin producing bacteria has been reported and some examples include Vibrio cholerae and Shewanella colwelliana, where they produce pyomelanin due to the catabolism of tyrosine via the tyrosine degradation pathway. Marine bacteria such as Aeromonas salmonicida and Alteromonas nigrifaciens produces melanin in vivo with the help of the tyrosine precursor [22]. Moreover, Proteobacteria such as Marinomonas mediterranea and Actinobacteria such as Streptomyces kathirae and Streptomyces lusitanus DMZ-3 were also able to produce melanin. In V. cholera, melanin is induced in response to environmental conditions [23]. Naturally, melanin is not considered essential for growth and development of some microorganisms but are required to enhance their ability to compete and survive in unfavorable environmental conditions [21]. Melanin acts as a protective agent against thermal, desiccation, hyperosmotic shock, chemicals (oxidizing agents and heavy metals), and cell damage by solar UV radiation [19]. Moreover, in some pathogenic bacteria, melanisation becomes a virulence factor since melanin protects bacterial cells from defense mechanism in the infected host [18].

Violacein: Violacein is a water-insoluble violet or purple pigment found in Chromobacterium violaceum, first isolated from the Amazon River in Brazil [24]. Various studies have shown that violacein possesses different bio-activities such as antimicrobial, anticancer, antiviral and anti-ulcerogenic activities. It has also become a useful indicator of quorum sensing due to its ease of visualization [21]. In nature, violacein might be responsible for protection against UV radiation [22]. Pyocyanin: In nature, the human pathogen Pseudomonas aeruginosa can be found in terrestrial and aquatic environments, and it can cause serious infection. The most characteristic feature of P. aeruginosa is the production of the soluble pigment known as pyocyanin [20]. Pyocyanin is a blue green pigment and is composed of two subunits of N-methyl-1-hydroxyphenazine [21]. Studies have revealed that phenazines contributes to the virulence of P. aeruginosa and pyocyanin is the main phenazine produced by P. aeruginosa and has been shown to contribute to the unusual persistence of P. aeruginosa infections [22]. Even though P. aeruginosa is well known for its association with chronic infections in cystic fibrosis and wound infections due to burns, P. aeruginosa also can produce pigments with useful bio-activities. As mentioned earlier, besides as coloring agents, pigments have also been reported to possess various biological properties. Therefore, the following sections will focus on the studies and research done on therapeutic applications of bacterial pigments.

Materials and methods

Isolation and identification of pigmented bacterium

Samples were obtained from 15 m depth of the Caspian Sea (36.46°N 51.02°E). They were aseptically pooled and diluted up to 10^{-5}, and inoculated on the artificial sea water (ASW) agar medium containing (g l^{-1}): NaCl 10, $MgSO_4$ 0.52, $FeSO_4$ 0.01, $MgCl_2.6H_2O$ 0.19, $(NH_4)_2SO_4$, 0.98, $CaCl_2.2H_2O$ 0.19, KCl, 0.15, peptone 10, yeast extract 5, glucose 2 and agar 15. The pH was adjusted to 8, and the plates were kept at 20°C for at least 1 month. A red-pigmented bacterium designated as strain G20 was obtained in the isolation procedure. The extraction of genomic DNA, PCR amplification of 16S rRNA and phylogenetic analysis were carried out as described later.

Preparation of the pigment extract

The bacterial cells were grown in the ASW broth medium in a 1000-ml Erlenmeyer flask and kept in a

[DOI: 10.47176/sjmshm.6.1.1]

shaker incubator (150 rpm and 20°C) until the culture medium turned red. The bacterial cells were harvested following centrifugation at 4000 rpm for 20 min. The cell pellets were extracted with 95% (v/v) methanol until the pellets became colourless. The methanolic extract was collected and evaporated under reduced pressure below 40°C in a rotary evaporator (Heidolph, Germany), and then it was freeze-dried (Freeze dryer, Christ Alpha 1.2 LD Plus, Germany). Thin layer chromatography (TLC) was developed using silica plates (Merck, Darmstadt, Germany). The concentrated pigment was dissolved in a methanol solution, spotted on a silica gel sheet and developed with a mobile phase of ethanol: Diethyl ether (1:4 v/v) at room temperature in the dark. For preliminary chemical characterization, the UV-visible spectrum of the pigment was recorded between 200 and 800 nm, using methanol as a blank. High-performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA) consisting of a 1525 binary pump and a 2489 UV/vis detector set at 440 nm, was used. Separation was conducted on an InertSustainSwift C18 column (150 mm length, 46-mm internal diameter and 5- lm particle size) and the data were analyzed with the Breeze 2 software (Waters). Linear gradient elution chromatography was done using solvent systems A (methanol) and B (water) at ambient temperature. The gradient program used was as follows: initial, A–B (20:80, v/v), and 0– 60 min, linear change to A–B (80:20, v/v) at 1% methanol/ min gradient speed. The flow rate was kept constant at 1 ml min1, and 20 ll of the methanol-water (50%, v/v) extract of pure pigment was applied. Crocin—a wellknown plant carotenoid—was used as the reference pigment. Fourier transform infrared spectroscopy (FTIR) was measured in cuvettes with windows of calcium florid. The transformed specters were background-corrected for the solvent.

Enhancing pigment production

To increase the pigment production, amounts of different factors including the carbon source (1% w/v of glucose, fructose, maltose and starch), temperature (10–40°C), pH (6–9) and incubation time (0–120 h) were varied one at a time in an ASW basal broth medium (20°C, pH 8). Each subsequent factor was examined after taking into account the previously optimized factor(s). In each experiment, the absorbance of methanolic extract was measured at 490 nm, and the bacterial growth was determined by the measurement of the absorbance at 600 nm (Shimadzu, Japan).

Antioxidant activity

The antioxidant capacity of the pigment was studied following a method described by Blois (1958). Briefly, twofold serial dilutions of the pigment prepared in dimethyl sulfoxide were added to a 0.1 mM DPPH (1,1-diphenyl2-picrylhydrazyl) (Sigma, USA) methanolic solution, and then left in darkness at 30°C for 30 min. The absorbance of the resulting solutions was measured calorimetrically at 517 nm. A sample containing only the solvent and the DPPH without the pigment was used as a control. The scavenging effect on the DPPH was determined as follows:

DPPH scavenging effect (%) $=[(A_0 - A_1) / = A_0] \times 100$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the pigment extract.

Cytotoxic activity

Cell lines and cell culture KYSE-30 (a human esophageal squamous carcinoma cell line) cells were obtained from the Pasteur Institute (Tehran, Iran) and cultured in the Roswell Park Memorial Institute medium (RPMI 1640, Gibco, Scotland) with 10% fetal bovine serum (FBS, Gibco, Scotland). Human dermal fibroblasts cells as a gift from the Royan Institute (Tehran, Iran) were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, Scotland) and enriched with fetal bovine serum (FBS, Gibco, Scotland). Both cell lines were grown at 37°C in a humidified atmosphere of 5% CO2 in the air. Cell viability assay Cell viability was determined by the MTT [3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. For this, 4000 KYSE30 cells and 8000 HDF cells were incubated in 96-well plates, in the absence (control) or presence (test) of various concentrations of the pigment in a final volume of 200 ll for 24, 48 and 72 h. A total of 20 ll of MTT (diluted in PBS) was added to each well, and after 4 h of incubation, the precipitated purple MTT formazan crystals were dissolved in 150 ll DMSO, and the absorbance was recorded at 540 nm (Stat Fax, America). The cell viability was measured by the following formula:

Viability $=[(A_T/A_C] \times 100$

where AC was the absorbance of the control reaction and AT was the absorbance in the presence of the pigment extract. Treated and control cells were observed under a light inverted microscope (Olympus, Japan) for monitoring the morphological changes after 24, 48 and 72 h.

[DOI: 10.47176/sjmshm.6.1.1]

Antibacterial activity

The effects of the pigment on the growth of the selected microbial strains were evaluated in a liquid Mueller Hinton medium using the microbroth serial dilution approach (Kim et al. 2007). Staphylococcus aureus (PTCC1431), Bacillus subtilis (PTCC1023), Pseudomonas aeruginosa (PTCC1074) and Escherichia coli (PTCC 1330) were selected as reference strains. Microbial growth was determined by measuring the turbidity at 600 nm.

Data analysis

All quantitative experiments were repeated at least three times. The data analysis was conducted using GraphPad Prism version 6.0 and the IC50 and EC50 values were obtained from nonlinear regression.

Results

Isolation and identification of pigmented bacterium

Strain G20 was obtained from 15 m depth of Caspian Sea coastal water. The novel strain was Gram-negative, nonmotile and rod shaped. The cells of strain G20 formed convex and round colonies with an entire edge on the solid ASW medium after 2 days (Fig. 1a). The preliminary chemical characterization of the pigment extract is presented in Fig. 1b–d. As seen in Fig. 1b, the UV–Vis spectrum of pigment showed the maximum absorption at 491 nm, which is in the range of reports for various carotenoids . The FTIR spectrum for pigment (Fig.1c) showed characteristic absorption frequencies of the carbonyl group at 1737 cm^{-1},alkene group at 1635 cm^{-1},aromatic ether group at 1264 cm^{-1},CH_2 (bending) at 1466 cm^{-1}, mono-substituted benzene group at 721 cm^{-1} and the hydroxyl group at 3000 cm^{-1}.Further signals were identified as CH_3/CH_2 stretch vibrations at 2962 cm^{-1} and 2851 cm^{-1}. The IR spectrum of the pigment extract is similar to the spectrum of purified lycopene. Comparing the HPLC chromatogram of the novel pigment solution with the chromatogram of the crocin solution (Fig. 1d) showed the major peak at the retention time of 30.536 min, followed three minutes later by a smaller but marked peak, which is comparable with crocin, a plant carotenoid that is found in the flowers of Crocus and Gardenia. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain G20 is a member of the genus Arthrobacter (Fig. 2). The closest relatives of strain G20 were Arthrobacter echini AM23^T, Arthrobacter agilis KCTC 3200^T and Arthrobacter pityocampae Tp2^T with a gene sequence similarities of 99.4%.

Antioxidant activity

The novel carotenoid pigment has the ability to act as an antioxidant, and its EC_{50} scavenging activity of DPPH was equal to 4.5 mg ml^{-1}. This amount is comparable to well-known antioxidants like β-carotene and α-tocopherol, for which the EC_{50} scavenging capacities are reported as 3.5 and 1.5 mg ml^{-} respectively.

Cytotoxic activity

As revealed by the MTT assay, the pigment extract had no detectable effects on the viability of the normal HDF cells (Fig. 4a,e) while it showed a moderate antitumour effect on the oesophageal cancer cells (Fig. 4b,e). The IC_{50} values of the pigment on the $KYSE_{30}$ cells were calculated as (μg ml^{-1}) 1321, 668 and 366 after 24, 48 and 72 h respectively. Microscopic observation revealed that after treatment with the pigment, cells turned to round shapes in comparison with control cells (Fig. 4c,d).

Antibacterial effects

The novel carotenoid-related pigment obtained in the current study did not display significant antibacterial activity. Only a weak activity against E. coli was observed at a high pigment concentration (MIC50 equal to 25 mg ml^{-1}) (Data not shown).

[DOI: 10.47176/sjmshm.6.1.1]

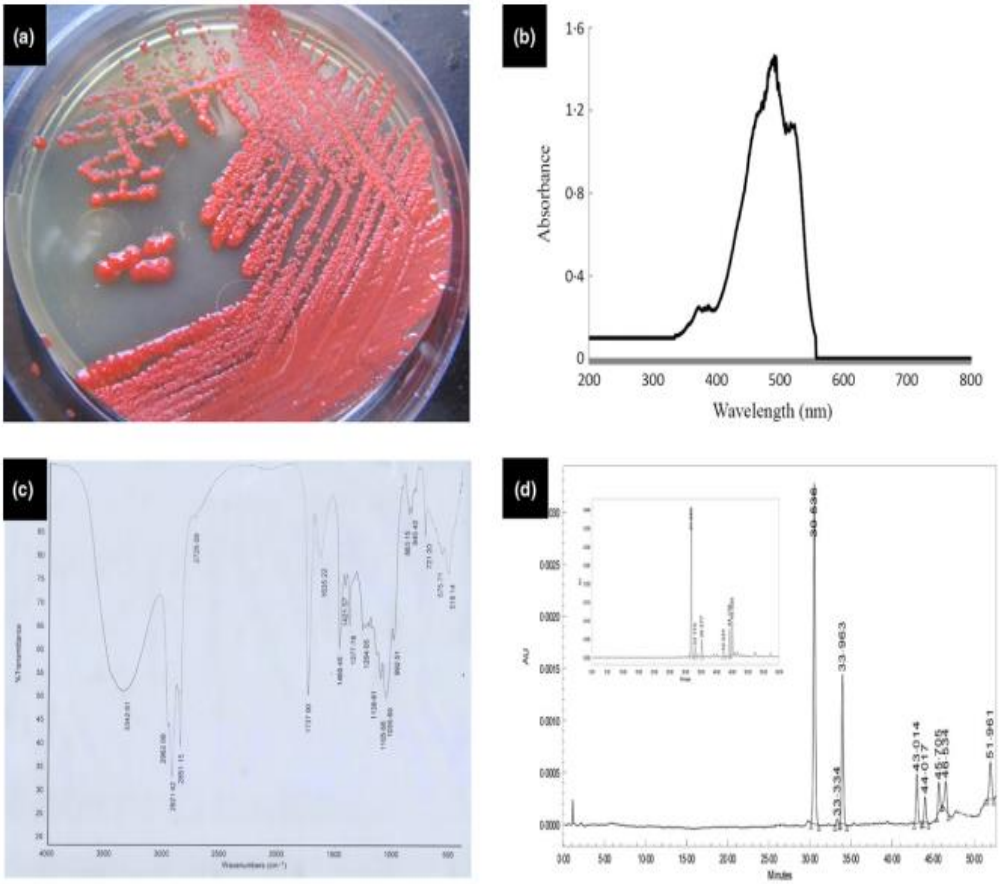

Fig 1. *Some characteristics of the novel pigment. (a) Pure culture of pigment producing Arthrobacter sp. G20 grown on ASW medium. (b) UV/vis spectrum of pigment. (c) FTIR spectrum of methanolic extract. (d) HPLC analysis of novel pigment in comparison to crocin (top-left corner). [Colour figure can be viewed at wileyonlinelibrary.com]*

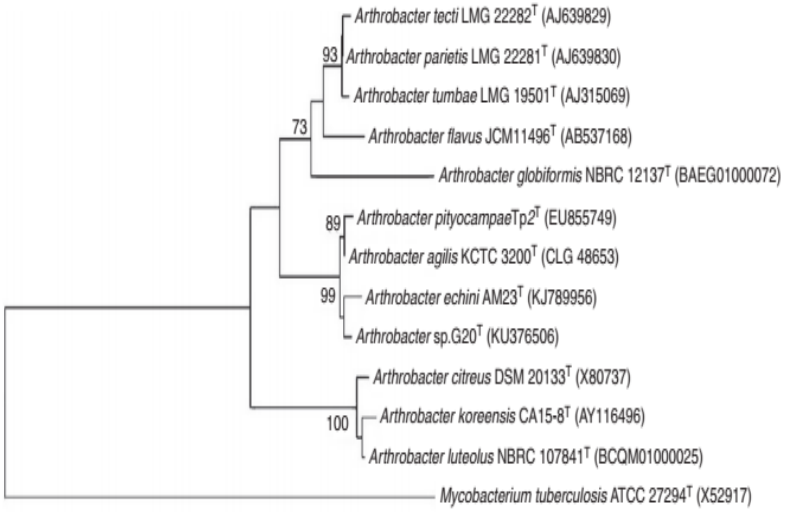

Fig 2. *Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing of the novel strain and other identified Arthrobacter.*

[DOI: 10.47176/sjmshm.6.1.1]

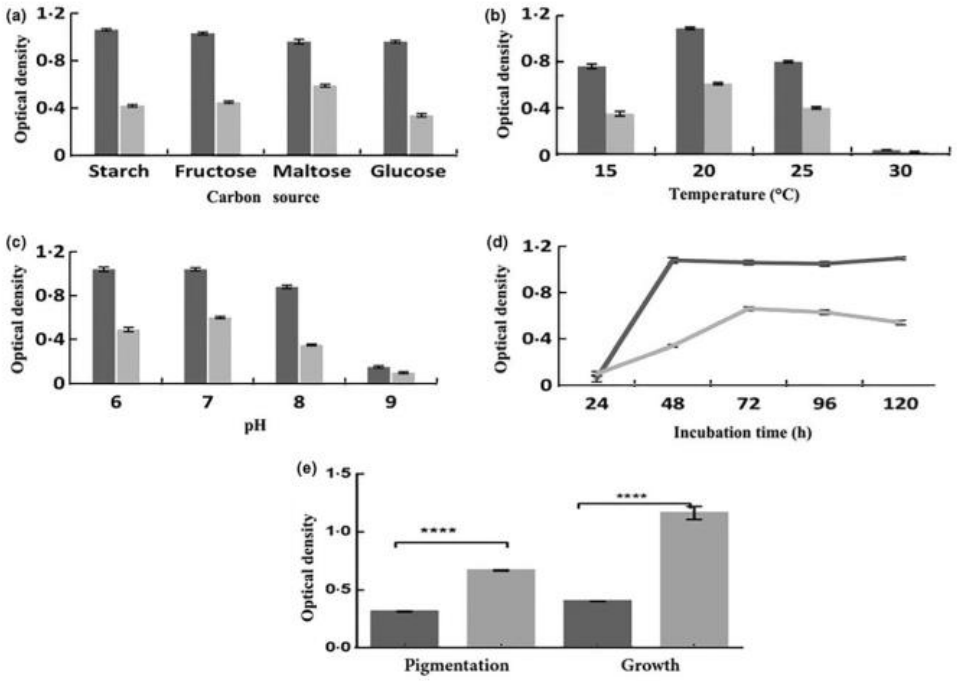

Fig 3. *Effects of different factors on the growth (dark grey) and pigment production (light grey) by Arthrobacter sp. G20. (a) Carbon source (20°C, pH = 8); (b) temperature (pH = 8); (c) initial pH and (d) incubation time. (e) Comparison of pigment production and bacterial growth before (dark grey) and after (light grey) optimization. ****P < 00001 (un-pair ANOVA with Tukey post hoc test).*

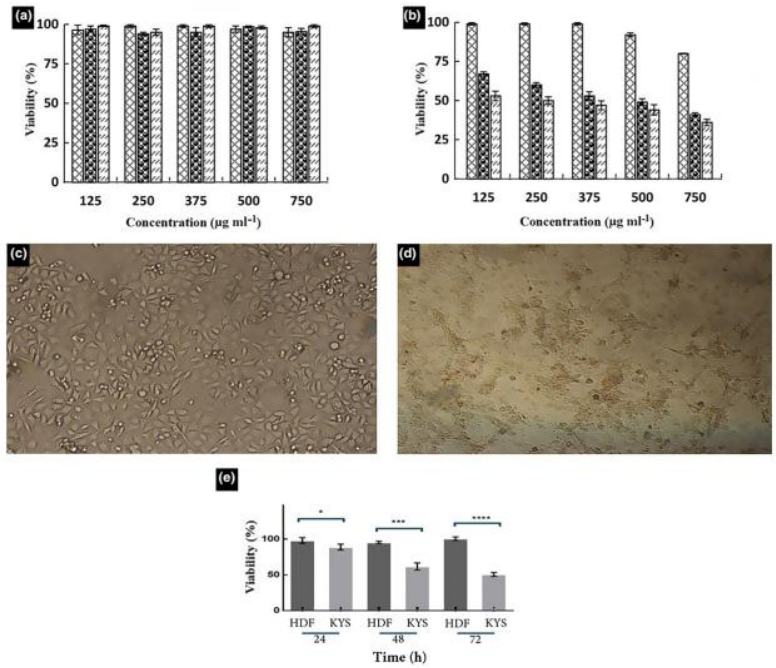

Fig 4. *Effect of red pigment on cell viability and morphology. (a) Effect on normal HDF cell viability after 24 (diamond), 48 (sphere) and 72 (diagonal) h. (b) Effect on cancerous KYSE30 cell viability after 24 (diamond), 48 (sphere) and 72 (diagonal) h. (c) Light microscopic images of nontreated KYSE30 cells. (d) Light microscopic images of treated KYSE30 cells with pigment (750 lg/ml) after 72 h. (e) Comparison the viability of HDF (dark grey) and KYS30 (light grey) cells after treatment by pigment extract. *P < 005, ***P < 001, ****P < 00001 (unpaired t-test). [Colour figure can be viewed at wileyonlinelibrary.com]*

[DOI: 10.47176/sjmshm.6.1.1]

Discussion

In this study, the biological activities of a novel pigment obtained from the marine Arthrobacter sp. were investigated. Based on the chemical characterization and compared to other known pigments, it was suggested as a member of carotenoids. The genus Arthobacter, is phenotypically heterogeneous, and its 89 species [24] have been isolated from various sources such as soil, fresh water, fermented food, clinical specimens, paintings, air, ocean sediment, etc. Except for some species like A. scleromae, A. histidinolovorans and A. albus, the bacteria of this genus produce pigments with a great variety of hues and structures [25]. Riboflavin (yellow), indigoidine (blue), indochrome (blue) and porphyrins (red) are examples of various structures observed in this genus [26]. A. roseus and A. agilis are two Arthrobacter species isolated from cold environments, which contain red carotenoid pigments [27]. It was stated that pigmentation is responsible for the cold adaptation of these psychrotrophic bacteria through cell membrane stabilization and radiation protection [28]. Although carotenoid production is demonstrated in this genus, there are no reports so far about the biological activities of carotenoids obtained from Arthrobacter spp. As regards the enhancement of the pigment production by optimization of the culture condition, maltose was the most suitable carbon source in comparison to glucose and starch. As reported previously psychrophilic Arthrobacter, unlike mesophilic ones, could not hydrolase starch [29] which explains why starch is not a suitable carbon source for pigment production by this psychrophilic strain. Our results indicated that more complex carbon sources (like maltose) are more desirable than simple monosaccharide (glucose and fructose) for the production of this secondary metabolite. We also observed the best bacterial growth at 20°C and the best pigment production yield (ratio of pigment production to biomass) at 15°C. Free radicals—which contain a single unpaired electron in their molecular structures—are generated in the human body for various endo/exogenous reasons. These molecules adversely alter cellular lipids, proteins and DNA, and trigger a number of human diseases like cancers. Antioxidants like carotenoids are compounds that donate an electron to rampaging free radicals and neutralize them. Therefore, they are well-known as preventing agents for cancers and other diseases in the pharmaceutical and food industries. The antioxidant capacity of our novel pigment is comparable to well-known antioxidants like b-carotene and a-tocopherol. These data suggest that Arthrobacter spp. are interesting sources for the bacterial-based antioxidant production. There are remarkable reports about the role of antioxidants in lowering the risk of a variety of cancers. However, some scientists have argued about their role in cancer treatment. This is because of the fact that tumor development is accompanied by decreasing the intracellular reactive oxygen species (ROS). In cancerous cells, ROS acts as an apoptosis-promoting agent and the excess of antioxidants can block these cancer-preventive mechanisms and develop cancer [27-28]. Recent studies demonstrated that the alterations of redox-active iron metabolisms in cancer cells in comparison to normal cells are responsible for the specific activity of ascorbic acid (antioxidant agent) against no small-cell lung cancer (NSCLC) and glioblastoma (GBM) cells. It is understood from this example that an excess of antioxidants can also kill cancer cells but by using a different mechanism [28]. There are some other reports that indicated that carotenoids harbour both anticancer and antioxidant activity, and could promote the apoptosis cascade and kill the tumor cells [29] For instance, Lycopene suppresses the development of gastric cancer and induces apoptosis in smoke-induced lung cancer. In the current study, we observed that the treated cancerous cells were rounded, which is a morphological characteristic of apoptotic cells. However, the exact mechanism of its action remains to be clarified. The tested pigment did not show remarkable antibacterial activity. There are no uniform reports about the antibacterial activity of carotenoids. The differences in their activities may be attributed to the various pigment formulas, as well as different bacterial cell structures. Some pigments could inhibit the growth of bacteria like Bacillus cereus, but others did not affect B.subtils and Salmonella typhimurium In conclusion, a novel marine and cold adaptive Arthrobacter sp. was isolated from the Caspian Sea. This strain produced a nonwater-soluble red pigment. The chemical characterization of the pigment suggested that it belongs to carotenoids. The pigment showed remarkable antioxidant activity. The specific antiproliferating effect against a KYSE30 cancerous cell line in comparison with normal HDF cells was observed. This is the first report on the pharmaceutical potential of carotenoid pigments from Arthrobacter spp. However, further studies are required to determine the

[DOI: 10.47176/sjmshm.6.1.1]

structure and the exact mechanisms involved in the function of this pigment.

References

1. Tibor, C. (2007). Liquid Chromatography of Natural pigments and synthetic dyes. *J. Chromatography Library*, *71*, 11-19.
2. Mapari, S. A., Nielsen, K. F., Larsen, T. O., Frisvad, J. C., Meyer, A. S., & Thrane, U. (2005). Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. *Current Opinion in Biotechnology*, *16*(2), 231-238.
3. Cristea, D., & Vilarem, G. (2006). Improving light fastness of natural dyes on cotton yarn. *Dyes and pigments*, *70*(3), 238-245.
4. Räisänen, R., Nousiainen, P., & Hynninen, P. H. (2002). Dermorubin and 5-chlorodermorubin natural anthraquinone carboxylic acids as dyes for wool. *Textile research journal*, *72*(11), 973-976.
5. Parekh, S., Vinci, V. A., & Strobel, R. J. (2000). Improvement of microbial strains and fermentation processes. *Applied microbiology and biotechnology*, *54*, 287-301.
6. Ahmad, W. A., Wan Ahmad, W. Y., Zakaria, Z. A., Yusof, N. Z., Ahmad, W. A., Ahmad, W. Y. W., ... & Yusof, N. Z. (2012). *Application of bacterial pigments as colorant* (pp. 57-74). Springer Berlin Heidelberg.
7. Joshi, V. K., Attri, D., Bala, A., & Bhushan, S. (2003). Microbial pigments.
8. Venil, C. K., & Lakshmanaperumalsamy, P. (2009). An insightful overview on microbial pigment, prodigiosin. *Electronic Journal of Biology*, *5*(3), 49-61.
9. Parmar, M., & Phutela, U. G. (2015). Biocolors: the new generation additives. *Int J Curr Microbiol Appl Sci*, *4*(7), 688-694.
10. Nakashima, T., Kurachi, M., Kato, Y., Yamaguchi, K., & Oda, T. (2005). Characterization of bacterium isolated from the sediment at coastal area of Omura Bay in Japan and several biological activities of pigment produced by this isolate. *Microbiology and immunology*, *49*(5), 407-415.
11. Kamla, M., Jayanti, T., & Sneh, G. (2012). A review on microbial pigment. *Int J Microbial Res Technol*, *1*(4), 361-365.
12. Aberoumand, A. (2011). A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World Journal of Dairy & Food Sciences*, *6*(1), 71-78.
13. Bener, M., Özyürek, M., Güçlü, K., & Apak, R. (2010). Polyphenolic contents of natural dyes produced from industrial plants assayed by HPLC and novel spectrophotometric methods. *Industrial crops and products*, *32*(3), 499-506.
14. Jensen, M. B., López-de-Dicastillo Bergamo, C. A., Payet, R. M., Liu, X., & Konczak, I. (2011). Influence of copigment derived from tasmannia pepper leaf on davidson's plum anthocyanins. *Journal of food science*, *76*(3), C447-C453.
15. Durgade, P. S., Jadhav, P. P., Shaikh, T. I., & Trivedi, M. M. (2022). Pigment producing bacteria and its application in various field.
16. Sivakumar, V., Vijaeeswarri, J., & Anna, J. L. (2011). Effective natural dye extraction from different plant materials using ultrasound. *Industrial Crops and Products*, *33*(1), 116-122.
17. Soliev, A. B., Hosokawa, K., & Enomoto, K. (2011). Bioactive pigments from marine bacteria: applications and physiological roles. *Evidence-based Complementary and Alternative Medicine: ECAM*, *2011*.
18. Kong, J. M., Chia, L. S., Goh, N. K., Chia, T. F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry*, *64*(5), 923-933.
19. Kim, H. W., Kim, J. B., Cho, S. M., Chung, M. N., Lee, Y. M., Chu, S. M., ... & Lee, D. J. (2012). Anthocyanin changes in the Korean purple-fleshed sweet potato, Shinzami, as affected by steaming and baking. *Food chemistry*, *130*(4), 966-972.
20. Youdim, K. A., McDonald, J., Kalt, W., & Joseph, J. A. (2002). Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *The Journal of nutritional biochemistry*, *13*(5), 282-288.
21. Wang, J., & Mazza, G. (2002). Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor α in LPS/IFN-γ-activated RAW 264.7 macrophages. *Journal of Agricultural and Food Chemistry*, *50*(15), 4183-4189.
22. Kim, H., Han, S., Lee, C., Lee, K., Park, S., & Kim, Y. (2003). *U.S. Patent No. 6,638,968*. Washington, DC: U.S. Patent and Trademark Office.
23. Campas, C., Dalmau, M., Montaner, B., Barragán, M., Bellosillo, B., Colomer, D., ... & Gil, J. (2003). Prodigiosin induces apoptosis of B and T cells from B-cell chronic lymphocytic leukemia. *Leukemia*, *17*(4), 746-750.
24. Yada, S., Wang, Y., Zou, Y., Nagasaki, K., Hosokawa, K., Osaka, I., ... & Enomoto, K. (2008). Isolation and characterization of two groups of novel marine bacteria producing violacein. *Marine*

[DOI: 10.47176/sjmshm.6.1.1]

biotechnology, *10*, 128-132.
25. Matz, C., Deines, P., Boenigk, J., Arndt, H., Eberl, L., Kjelleberg, S., & Jürgens, K. (2004). Impact of violacein-producing bacteria on survival and feeding of bacterivorous nanoflagellates. *Applied and Environmental Microbiology*, *70*(3), 1593-1599.
26. Ferreira, C. V., Bos, C. L., Versteeg, H. H., Justo, G. Z., Durán, N., & Peppelenbosch, M. P. (2004). Molecular mechanism of violacein-mediated human leukemia cell death. *Blood*, *104*(5), 1459-1464.
27. Kodach, L. L., Bos, C. L., Durán, N., Peppelenbosch, M. P., Ferreira, C. V., & Hardwick, J. C. (2006). Violacein synergistically increases 5-fluorouracil cytotoxicity, induces apoptosis and inhibits Akt-mediated signal transduction in human colorectal cancer cells. *Carcinogenesis*, *27*(3), 508-516.
28. Sánchez, C., Braña, A. F., Méndez, C., & Salas, J. A. (2006). Reevaluation of the violacein biosynthetic pathway and its relationship to indolocarbazole biosynthesis. *Chembiochem*, *7*(8), 1231-1240.
29. Nakamura, Y., Sawada, T., Morita, Y., & Tamiya, E. (2002). 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. *Biochemical Engineering Journal*, *12*(1), 79-86.

SJMSHM

Citation: Sabouri Z, Ghorbani P. Investigating the Antioxidant, Cytotoxic and Antimicrobial Effects of a Red Pigment Obtained from Marine Bacterial Strain. SJMSHM, 2024; 6(1):1-11.

https://doi.org/10.47176/sjmshm.6.1.1

[DOI: 10.47176/sjmshm.6.1.1]