RESEARCH ARTICLE

Creation of a violacein pigment hybrid with silver and titanium dioxide nanoparticles to produce multifunctional textiles with antimicrobial properties

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ABSTRACT

Objective(s): Nowadays, with the increase of bacterial resistance to antibiotics and the number of death-related nosocomial infections, in addition to observing health protocols, the production and spread of these infectious agents could be prevented by producing antimicrobial fabrics for hospital textiles.

Methods: In this study, Janthinobacterium lividum was grown in the culture medium with suitable pigment production conditions, and then the produced violacein pigment was extracted from the culture media. The dyed fabrics were coated with two types of nanoparticles that are widely used and suitable for hospital usage. The two nanoparticles include silver nanoparticles that had antibacterial activity and titanium dioxide nanoparticles that possess anti-stain and self-cleaning properties. The fabrics were then examined by scanning electron microscopy and X-ray energy scattering analysis, and micrographs and energy dispersive X-Ray (EDAX) analysis were obtained from these samples. In order to analyze the structure of violacein and confirmation of dyeing, as well as proving the hybridization of the dye and nanoparticles through functional groups, Fourier transforms infrared spectroscopy (FTIR) was used. The antibacterial activity of the fabrics was also evaluated.

Results: Scanning electron microscopy (SEM) and EDAX methods showed that these nanoparticles were well-positioned on textile fibers, and the size and distribution of nanoparticles were determined. FTIR spectroscopy showed that silver and titanium dioxide nanoparticles formed a hybrid with violacein. Also, the antibacterial properties of dyed fabrics, along with hybrid fabrics, were investigated. The results indicated that the fabric dyed with violacein had special antibacterial activity, which increased with the degree of hybridization with nanoparticles.

Conclusions: The production of a natural antibacterial dye by employing biotechnological techniques with therapeutic properties could pave the way for designing new antibacterial fabrics for hospitals to overcome bacterial resistance.

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INTRODUCTION

Violacein is a purple-colored pigment produced in numerous gram-negative bacteria, inclusive of *Janthinobacterium lividum* [1]. It has been reported that this pigment has immunomodulatory, anti-fever, and antioxidant properties and could act as a tranquilizer agent [2]. Some studies that violacein can interfere with the central molecular pathways involved in the development of colorectal cancer and exhibit therapeutic effects on the pathogenesis of this type of cancer [3]. Violacein also possesses antibacterial properties against *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megatherium*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, and other bacteria [4]. Natural pigments such as violacein show less toxicity and greeter a disintegration level than that of synthetic pigments and often used as preservatives in food. Violacein is also effective in wound healing and has immunomodulatory, analgesic, and antipyretic properties, such as antioxidant properties [4]. Fabric dyeing using biological dyes, such as prodigiosin or violacein, with antimicrobial properties, is an attractive prospect for solving the environmental and health problems caused by the fabric dyeing industry. It has been shown that textile fibers stained with violacein also have antibacterial, antioxidant, and therapeutic properties [5]. In the field of medical textiles, the use of textiles with antimicrobial potential is of special importance. Surgical sutures and antimicrobial dressings are among the most broadly used products in this field. Medical textiles can be considered a marked reservoir of pathogens that are involved in the development of nosocomial infections, and these textiles play an essential role in transmitting these pathogens [6]. The scrubs of nurses and physicians act as protective clothing to minimize the risk of infection. This uniform can be an important factor in the spread of nosocomial infections, and its consequences affect not only staff and nurses but also patients. Sometimes hospital textiles are used to clean surfaces, which can be disposable or reusable textiles [7]. Ideally, these textiles used for cleaning should be able to remove contaminations from the surface well and then easily wash and clean the contaminations during a rinsing process. Recently, microfiber fabrics have been introduced to hospitals, which are heavily marketed. One of the common significant stages for the treatment of fabrics is to get the favorite dyeing qualities with antimicrobial activity [6]. Also, recently in nanomedicine, instead of using chemicals, the use of nanoparticles with antimicrobial properties is an appropriate method for the production of antimicrobial fabrics. Nano-antimicrobial materials, such as Titanium dioxide (Tio2) metal nanoparticles, including silver, gold, zinc oxide, and copper nanoparticles, are used in the textile industry [8, 9]. These nanoparticles have properties such as self-cleaning, disinfection, protection from ultraviolet radiation, electric conductivity, and burning resistance. Silver is an antimicrobial agent that is less dangerous in comparison with organic substances and has less harmful effects on cells [10]. Its therapeutic properties have been proven against a wide range of microorganisms. Silver compounds even prevent the formation of bacterial biofilms. The advantages of silver ions include non-toxicity, biocompatibility, high hydrophobicity, structural complexity, antimicrobial activity, and acceptable solubility in water and other solvents. Due to the high potential of the application of nanomaterials in the textile industry, the production of high-performance fabrics, such as antimicrobial fabrics, has been facilitated. Titanium dioxide nanoparticles also have unique properties in a wide range of scientific fields. In this regard, the production of textiles with self-cleaning properties is proposed by bleaching stains in the presence of sunlight by this nanoparticle [8]. This nanoparticle also possesses high antibacterial and antifungal activity. Because nanoparticles have a higher surface area per unit volume, their antibacterial activity is higher. Moreover, titanium dioxide nanoparticles have a positive effect on fabric wrinkles. These particles have a good coating and are non-toxic and also have many applications due to their stability [11].

The present study aimed to produce and in-vitro extraction of violacein from *J. lividum* for fabric dyeing, as well as the creation of a pigment hybrid with two types of silver and titanium dioxide nanoparticles for the production of fabrics with multifunctional properties. Also, the antibacterial activity of the produced fabrics was assessed alone and in hybrid forms. After the staining process, the fabric is washed, and then silver nanoparticles are added. Also, the nano-property in nano-fabrics is a surface property that is decreased following the washing procedures. Consequently, it is better to produce nanofibers that give such properties to dyed fabrics. In this design, in addition to silver nanoparticles, titanium dioxide nanoparticles are also used and hybridized with violacein dye. In

Table 1. List of characteristics of the strains used

Name	Characteristics	Source
Janthinobacterium lividum	ATCC 12473 PTCC1735	Iranian Biological Resource Center
E.coli	ATCC35218 PTCC1399	Microbial Collection of Shahrekord University
Staphylococcus aureus	ATCC 6538 PTCC1112	Microbial Collection of Shahrekord University
bacillus cereus	ATCC 11778 PTCC1015	Microbial Collection of Shahrekord University

Table 2. The constituents of the culture medium optimized for dye production

Constituents	Amounts
Nutrient broth culture medium	8 gr
Tryptophan	1 %
Glycerol	1%
Magnesium Sulfate	1%
Distilled water	1 liter
pH	7.4± 0.2

addition to the fact that the antibacterial property of the produced hybrid is expected to be much higher, other properties, such as self-cleaning and anti-wrinkle, are added. So, since these textiles are used for medical purposes, gaining such properties would be significant. Self-cleaning property is associated with less detergent consumption and very useful in reducing sewage. In this study, for the first time, the pigment fixation with the two types of nanoparticles was performed.

MATERIALS AND METHODS

Bacteria used, culture media and solutions required for pigment production and microbiological evaluations

The strains used in addition to the main strain (row 1), prepared for pigment production, were used from the other 3 strains for antibacterial analyses (Table 1).

Nutrient broth, Agar, Mueller-Hinton agar, and TSB (Trypticase soy broth) were used for bacterial growth and biological tests, and the optimized culture medium depicted in Table 2 was used to produce the desired dye [12, 13].

Dye extraction method

Extraction of dye from the broth culture medium

To extract the dye from *J.lividum* cultivated in broth medium, after bacterial culture and incubation time for one week and when the optical density reached the highest point at 585 nm, the extraction operation was performed. Erlenmeyer flakes containing 100 ml of enriched broth nutrient medium were inoculated with 1% bacteria. The Erlenmeyer flasks containing dyed surface biofilm were incubated for a week without shaking and used for pigment extraction. When the absorption reached the desired level, the Erlenmeyer flasks were removed, and the contents were divided into 50 ml Falcons. Then, the Falcons were at 8000 rpm for 15 minutes at 4 ° C to remove the precipitates. According to the reference method, ethanol is added per gram of mass at a ratio of 10:1, to extract the dye. Finally, 50 ml of ethanol was added to the resulting precipitates and vortexed [12]. The Erlenmeyer flasks were then transferred to a sonic bath and sonicated at 40 Hz for 20 minutes. Next, the samples were centrifuged at 13000 rpm at 9°C for 15 minutes. After the end of the procedures, the supernatant containing the dye solution was removed [12].

Extraction of dye from the solid culture medium

In order to extract the dye from the microbial biomass resulting from strain culture on a nutrient agar medium in disposable plates, when the incubation period was over, the plates were removed. Using a spatula, the surface biomass of the medium, which was completely dyed and thick, removed and poured into a glass plate. This glass container was placed in the oven at 60 °C for 24 hours to dry the mass and eliminate moisture. Then, the container was removed, the dried mass adhered to the bottom of the glass container was shaved, poured into a mortar, and the resulting biomass was well-grinded. Afterward, 10 ml of 100% ethanol per gram of biomass powder was added to Erlenmeyer flasks, and they were placed in an ultrasonic bath at 40 Hz for 20 minutes, and sonication was performed. Upon completion, Erlenmeyer flasks were removed, and the white residue of the killed bacteria was visible in the dye solution. These solutions were transferred to the Falcons, specified for refrigerated centrifugation, and centrifuged, similar to the step carried out for the liquid extraction. Finally, the colored solution was isolated from the white sediment [14].

Preparation of dye powder

In order to prepare the dye powder, the dye solutions were transferred to a rotary instrument, and after 60 minutes, ethanol was separated from the dye and collected in a device tank.

Fabric dyeing method

Dyeing fabrics with pure powder dye

The viscose fabric was cut into 10×10 cm pieces and weighed. Then, 2% of the weight of the dye powder fabric was weighed, and the volume of the dyeing bath was considered L: R ratio (50:1). It should be noted that the ratio of water to ethanol is 1:1. The dye powder was first added to ethanol, and then water was added to reach proper volume. The resulting colored solution was adjusted to a pH value range of 10. Then, 20% of the weight of the fabric, NaCl was used. The colored solution was placed in a water bath at 100 °C for 15 minutes until the temperature reached 40 °C. The pre-soaked cloths were then added to the bath, and the temperature gradually increased to 80 °C. During the dyeing operation, Erlenmeyer flasks were constantly agitated while containing the dye solution, and the temperature was controlled. After 60 minutes, the fabrics were taken out of the dye bath and poured into an Erlenmeyer flask containing cold water [2, 15].

The dyeing process by bacterial culture with fabric

The Erlenmeyer flask was prepared with 100 ml of optimized broth nutrient medium. Three pieces of viscose fabric with dimensions of 10×10 cm were cut and weighed, all three of which were approximately equal in weight. The fabrics were washed and placed in Erlenmeyer flakes, and the Erlenmeyer flask containing the culture medium and fabrics was autoclaved. After this process and cooling of the culture media, 1% of the bacterial suspension, equal to 1ml, was inoculated with a sampler under aseptic conditions. Erlenmeyer flasks were incubated at 25 °C for one week. After incubation of cultured fabrics, the bacteria were removed and shaken after one week. The dyed biofilm was created on the surface of the fabrics while the culture medium was purple. The pH values of Erlenmeyer flasks were measured. The absorption of the culture medium was then read using a spectrophotometer. Using a pair of forceps, the fabrics were removed from the culture medium, and the fabrics were rinsed by the washing solution and then air-dried [16].

Dyeing with microbial biomass

The dry and powdered biomass (2%) that was well ground with a ceramic mortar was used for the dyeing process according to the on-weight-fabric (OWF) dyeing protocols. A piece of plain viscose fabric with dimensions of 10 × 10 was cut and weighed, which was equal to 2 grams. Regarding this weight of the fabric, the required amounts of dye and dye bath were determined, and the volume of the bath prepared with ethanol and water at a ratio of 1:5. First, the dye powder was mixed with ethanol and dissolved, then water was added to this volume. The pH values of the dye solution were adjusted according to the reference using 1M NaOH and 1M HCL at a range of 10, and then the resulting dye solution was placed on a pre-heated water bath. After 15 minutes, the dye bath temperature reached 40°C, during which time it was stirred frequently. The fabrics were pre-soaked twice in water for 30 minutes, then the dye was added to the bath, and the temperature was gradually raised to 80 °C. It remained in this state for 60 minutes, and the temperature was checked regularly. The solution was agitated until the dye solution stained all parts of the fabrics. The fabrics were then removed from the dye bath and placed in a container filled with double distilled water that had the same temperature as the dye solution. It was then poured into the washing solution containing Triton X100 and rinsed well. Then, they were washed with distilled water and dried at room temperature [16].

The coating process of the dyed fabrics with silver and titanium dioxide nanoparticles

At first, a solution at a concentration of 20 ppm silver and 1% titanium oxide nanoparticles was prepared. For each 1 gram of the fabrics, 30 ml of

Table 3. Characteristics of silver and titanium dioxide nanoparticles used in this study, based on information from manufacturers

Silver nanoparticles characterization	
Molecular formula	Ag
Molecular weight (g/mol)	107.87
Form	Powder
Color	Gray
Morphology	Spherical
Crystal structure	Face-Centered Cubic (FCC)
Size range (nm)	10-60
Total impurity (%)	< 3 (X-ray fluorescence, XRF)
Melting point (°C)	961.78
Boiling Point (°C)	2162
Density (g/cm^3)	10.5
Solubility	Insoluble
Titanium dioxide nanoparticles characterization	
Purity	99.5%
Aerodynamic Particle Sizer (APS)	5nm
Specific surface area (SSA)	480-650 m^2/g
Color	white
Bulk density	0.12 – 0.18 g/cm^3
True density	3.9 g/cm^3
pH	5.25-7.3
Loss of weight in drying	6%
Loss of weight on ignition	12%

the colloidal solution was required. Therefore, the dyed fabric, dyed in previous steps and weighed about 2 grams, was used. At this stage, 60 ml of this colloidal solution at concentrations mentioned for silver and titanium oxide nanoparticles were added to the colloidal solution and transferred to an ultrasonic bath. The samples were sonicated at 40 °C for 30 minutes at 40 Hz. Then, the fabrics were taken out of the solution, placed in a tray, and transferred in an oven to dry (50 °C; 30 minutes) [17]. It should be mentioned that Silver nanoparticles were prepared from Tehran Armina Engineering Company and titanium dioxide nanoparticles from US Research Nanomaterials. characteristics of silver and titanium dioxide nanoparticles were presented in Table 3.

The analysis of antibacterial activity

Three pathogenic strains were used to evaluate the antibacterial properties of dyed fabrics and hybrid fabrics. The strains were common in nosocomial infections, including *Staphylococcus aureus, Escherichia coli*, and *Bacillus cereus*. The colony counting method was used to assess the antibacterial properties. In this method, the concentration of half McFarland was first prepared from each of the studied strains. For each strain, 3 Erlenmeyer flasks were chosen, including control Erlenmeyer, Erlenmeyer with dyed fabrics, and Erlenmeyer with hybrid fabrics. Each Erlenmeyer flask contained 30 ml of the TSB medium (8 ml of distilled water and 22 ml of the TSB solution) and 10 pieces of 1-cm fabrics. Erlenmeyer flasks were autoclaved while the fabrics were placed inside the flasks. Following the cooling down process performed beside the flame, each of the Erlenmeyer flasks were inoculated with 100 µl of the half-McFarland suspension and incubated at 37 °C for 24 hours [3].

Colony count method and preparation of dilution series

For each Erlenmeyer flask, 9 test tubes were prepared with 900 µl of sterile saline. The tubes were all numbered and clearly identified for each bacterial strain, and each Erlenmeyer flask. After incubation, which contained turbidity, 100 µl of the sample was taken from each Erlenmeyer flask and added to tube number 1. Then, the tube was shaken a little, and 100 µl was removed from the same tube and inoculated into the second tube. This process continued until the last tube to make serial dilutions. For each tube, a plate containing the trypticase soy agar (TSA) medium was prepared, and 100 µl of the

Table 4. The results of dilution assay and antimicrobial properties of dyed and hybrid fabrics

Strain/Numbers of bacteria in relation to the sample	Control	Dyed Fabrics	Hybrid Fabrics
Staphylococcus aureus	2.86×10^{11}	2.3×10^{6}	2.1×10^{5}
Escherichia coli	2.79×10^{11}	2.7×10^{9}	2.2×10^{8}
Bacillus cereus	2.75×10^{12}	2.75×10^{10}	2.8×10^{9}

suspensions (after serial dilutions) were removed from each tube and cultured on the plates. This inoculum suspension was applied throughout the surface culture plate. The plates were transferred to an incubator at 37 °C for 24 hours. The number of colonies was counted on each plate [3].

Optical stability test on dyed fabrics

In order to evaluate the optical stability of dyed fabrics, a sample of dyed fabrics was delivered to the Department of Textile Engineering at the Isfahan University of Technology, and the dyed fabrics were placed in the SUNSET instrument for 24 hours. Then, the degree of color change in dyed samples and their paleness were analyzed by the blue scale method. Optical stability was graded between 1 and 8, and a larger number denotes better stability.

Abrasion stability test of dyed fabrics

One of the quality control tests in textile is the fabric abrasion stability test, which also evaluates the abrasion stability of the dye on the fabric. The dyes used for staining the fabrics have different abrasion stability. In this test, which is performed with a grayscale, the machine applies force to the surface of the fabric by moving it back and forth, and its color changes are examined. For this test, the dyed fabrics are delivered to the Department of Textile Engineering at the Isfahan University of Technology. This stability was measured according to the National Standard of Iran, number 204-1695. This stability was rated between 1 and 5, and a larger number implies better stability.

Microscopic examination of EDAXSEM for the identification and positioning the elements of the composition of violacein dye and titanium dioxide nanoparticles

The fabrics dyed with violacein pigment and then hybridized with nanoparticles were examined by scanning electron microscopy and X-ray energy scattering analysis, and micrographs and the EDAX analysis were obtained from these samples. In the obtained images, each element can be recognized by a particular spectrum, and their position can be checked in comparison with each other at the sample level. In fact, the EDAX analysis was employed as a visual analytics tool for Scanning electron microscope (SEM). Each element has a unique peak and spectrum obtained during a micrograph [17]. Analyzes were performed by the device operator at Isfahan University of Technology. Samples were prepared according to the method of the same university based on the relevant references [18, 19].

Fourier transform infrared spectroscopy

In order to analyze the structure of violacein and confirmation of dyeing, as well as proving the hybridization of the dye and nanoparticles through functional groups, Fourier transforms infrared spectroscopy (FTIR) was used. The KBr tablet method was used for spectroscopy, and infrared spectroscopy analyses help to identify samples that are performed based on specific spectra of each element, which helps in the chemical identification of the samples. The FTIR uses infrared wavelengths at a range between 100-10000 cm^{-1} [14, 20].

RESULTS AND DISCUSSION

The analysis of antibacterial properties

Colony count test and preparation of dilution series

The results indicated that the dyed and hybrid fabrics had antibacterial properties and less turbidity than the control Erlenmeyer. In addition, the antimicrobial activity rate of these two products on bacterial pathogens was different. Qualitatively, the best antibacterial property was related to the strain of *Staphylococcus aureus*. The culture media containing fabrics had less turbidity compared with the control Erlenmeyer. The results are shown in Table 4.

The results of this experiment show that the antibacterial property of dyed fabrics is as good as that of hybrid fabrics, and the dye can even be used alone for dyeing fabrics and fibers with therapeutic and special applications. Therefore, it is feasible to eliminate nanoparticles whose safe

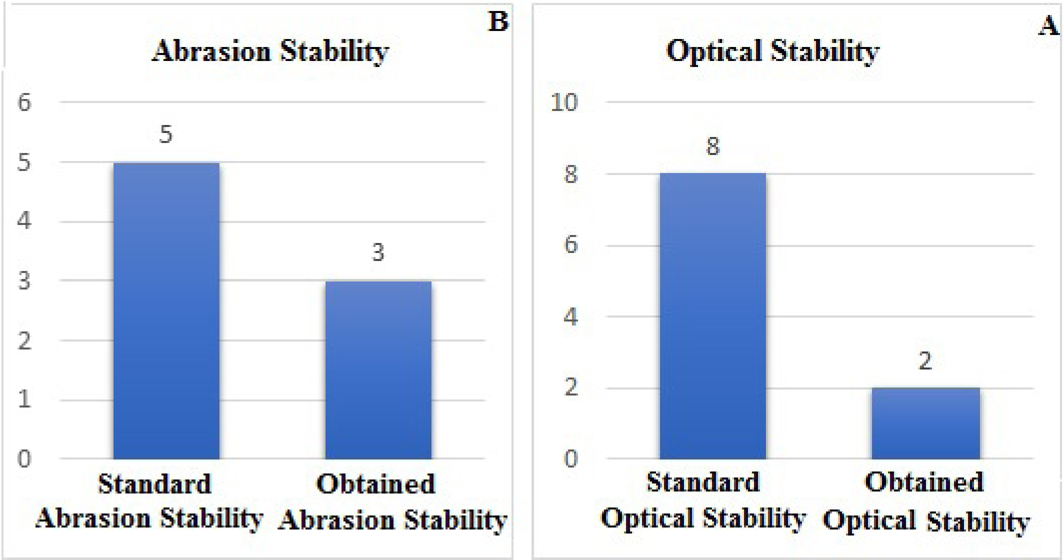

Fig. 1. Optical stability (A) and abrasion stability (B) diagrams of violet pigment dyed fabrics and comparison with the related standard diagram.

doses are not applicable in the industry. These produced fabrics have high antibacterial activity against some strains, such as *Staphylococcus aureus*, even in high concentrations of bacteria. This property is important because this strain is one of the dangerous pathogens of nosocomial infections. In the case of *Escherichia coli* as a gram-negative strain, the hybrid fabric is more effective than colored fabric at high concentrations. In Bacillus cereus, the antibacterial effects were observed in only low concentrations of bacteria. Thus, it is necessary to investigate the cause of a large difference in effectiveness between the two gram-positive strains, *Staphylococcus aureus* and *Bacillus cereus*.

Optical stability test on dyed fabrics

Due to the significance of physical tests on fabrics and their properties, the optical and abrasion stability analyses were carried out on the dyed fabric. The optical stability of fabrics, according to the blue scale, was estimated to be 2. This indicates the low optical stability of the dye. It should be noted that natural dyes have low light stability. Hospital fabrics are not exposed to sunlight; however, such a property should be taken into account for those fabrics exposed to sunlight. Optical stability diagram of violet pigment dyed fabrics and comparison with the related standard diagram was shown in Fig. 1A.

Abrasion stability test of dyed fabrics

The produced fabrics also had grade 3 stability in terms of abrasion stability according to the grayscale, indicating relatively good abrasion stability. According to this test, the appropriate abrasion stability in these fabrics make these fabrics suitable to be used for a longer period, and abrasion of fabrics with even skin surfaces will not lead to the separation of the hybrid formed on it. Abrasion stability diagram of violet pigment dyed fabrics and comparison with the related standard diagram was shown in Fig. 1B.

Microscopic examination of EDAXSEM for the identification and positioning of the elements of the violacein dye and titanium dioxide nanoparticles

The results showed the presence of silver and titanium nanoparticles with an average particle size of less than 90 nm (Fig. 2). It is clear that silver and titanium nanoparticles are distributed on the surface of dyed fibers (Fig. 2).

The EDAX analysis in Fig. 3 shows that the amount of silver and titanium oxide nanoparticles include 1% and 36% of the mass percentage. The EDAS mapping analysis was conducted for further confirmation of the presence of titanium and silver elements. The results demonstrated the presence of these elements (Fig. 4). According to this image, the proper distribution and dispersion of these elements on the surface of the fabric are well evident.

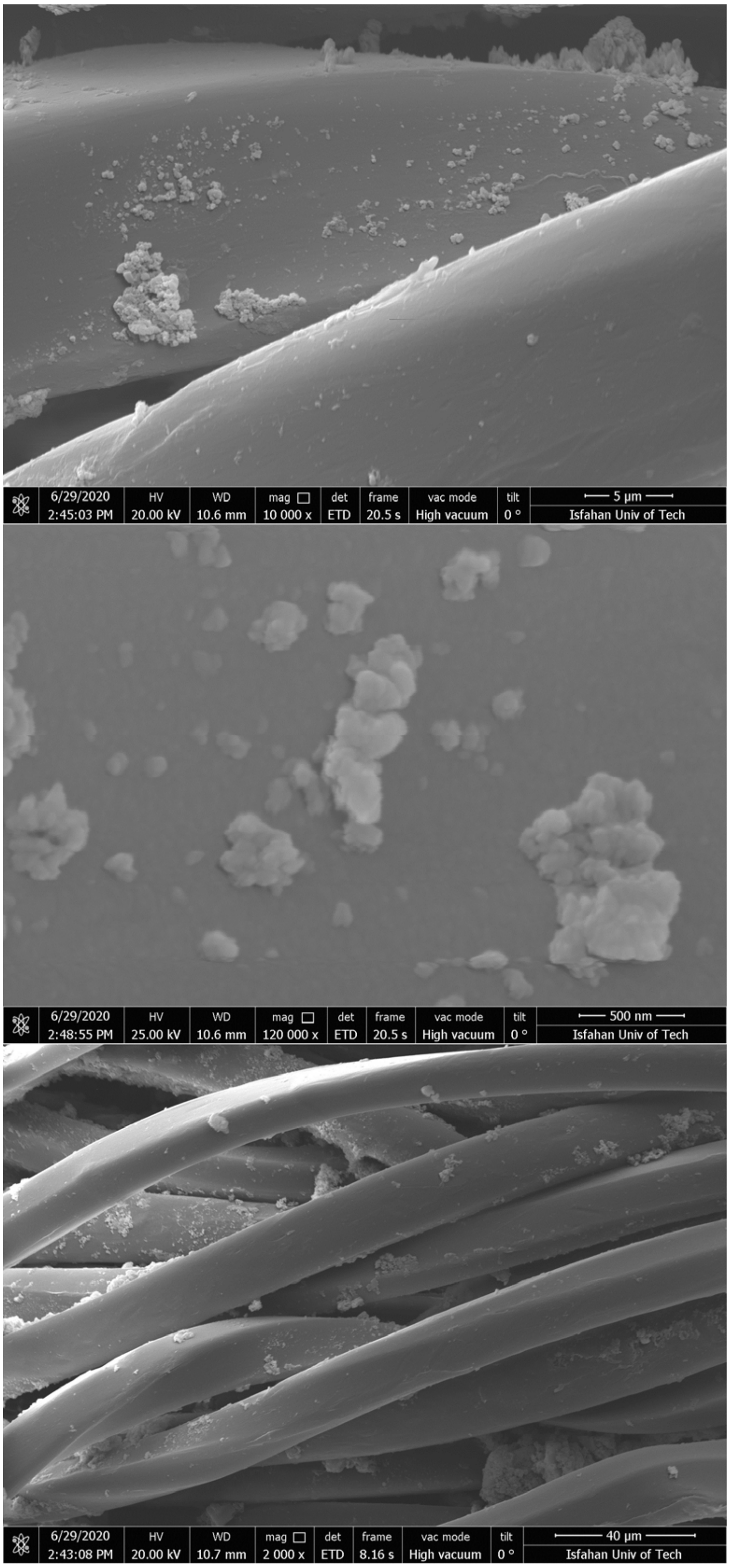

Fig. 2. SEM microscopic images of hybrid fabrics

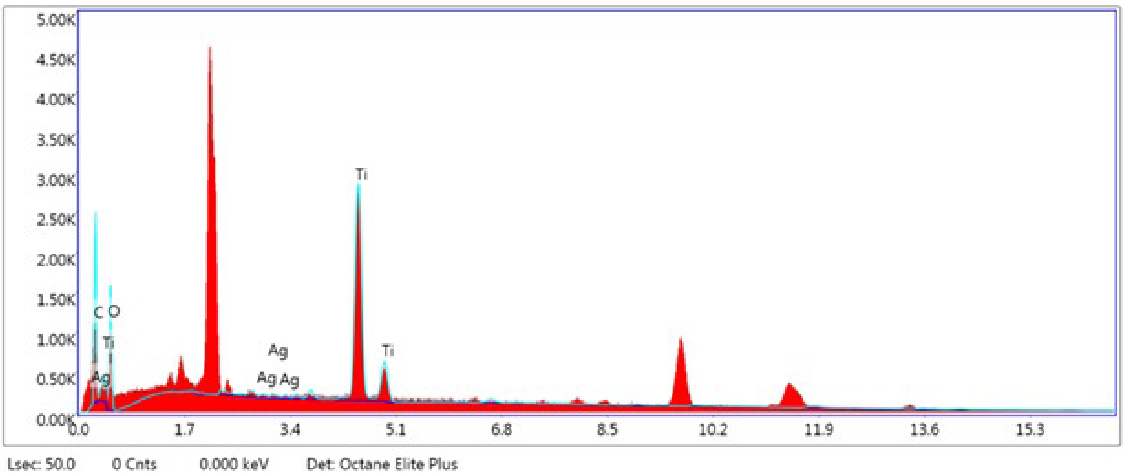

Fig. 3. The EDAX mapping analysis and confirmation of the presence of titanium dioxide and silver nanoparticles in hybrid fabric samples

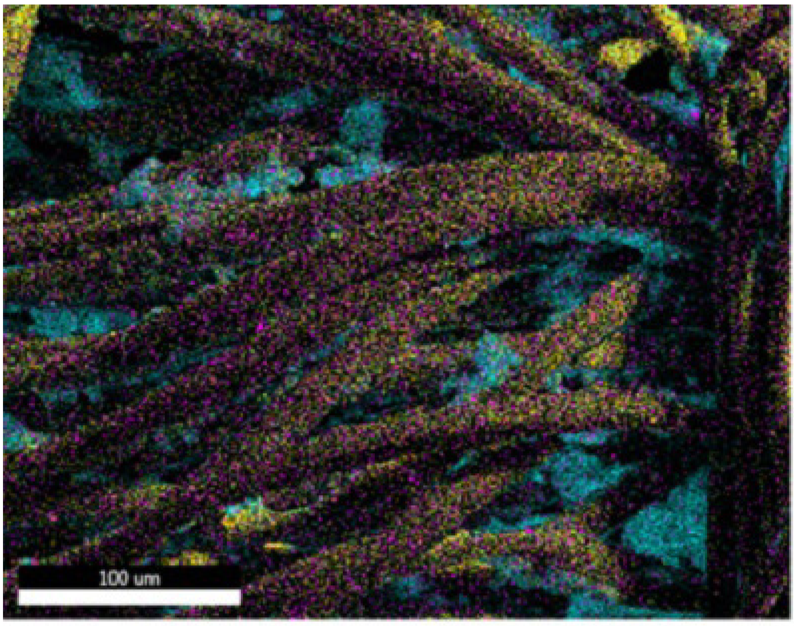

Fig. 4. Microscopic images of the mapping analysis of hybrid fabrics and the profile of nanoparticles, violacein dye, and fibers. The obtained images exhibit the pink color of silver nanoparticles and the blue color of titanium dioxide nanoparticles. Also, the yellow and green colors indicate the carbon and oxygen elements in the pigment composition, respectively.

FTIR analysis

The analysis of the relevant spectrum showed specific peaks at a wavelength of 1717 cm-1, which is assigned to the tensile vibrations of the carbonyl-amide group. A peak at 1240 cm-1 corresponds to the simple carbon-oxygen bond, while 1409 cm-1 is related to the carbon-carbon double bond. A wide peak appears at a wavelength of 3437 cm-1 is related to the overlapped bonds of OH and NH groups. Peaks appearing at wavelengths of 2907 m-1 and 2960 cm-1 are related to the tensile vibrations of C-H bonds. This structure clearly confirms the presence of the violacein pigment, and the spectrum obtained is similar to the reported structure of this pigment derived from the bacterium *J. lividum* (Fig. 5A).

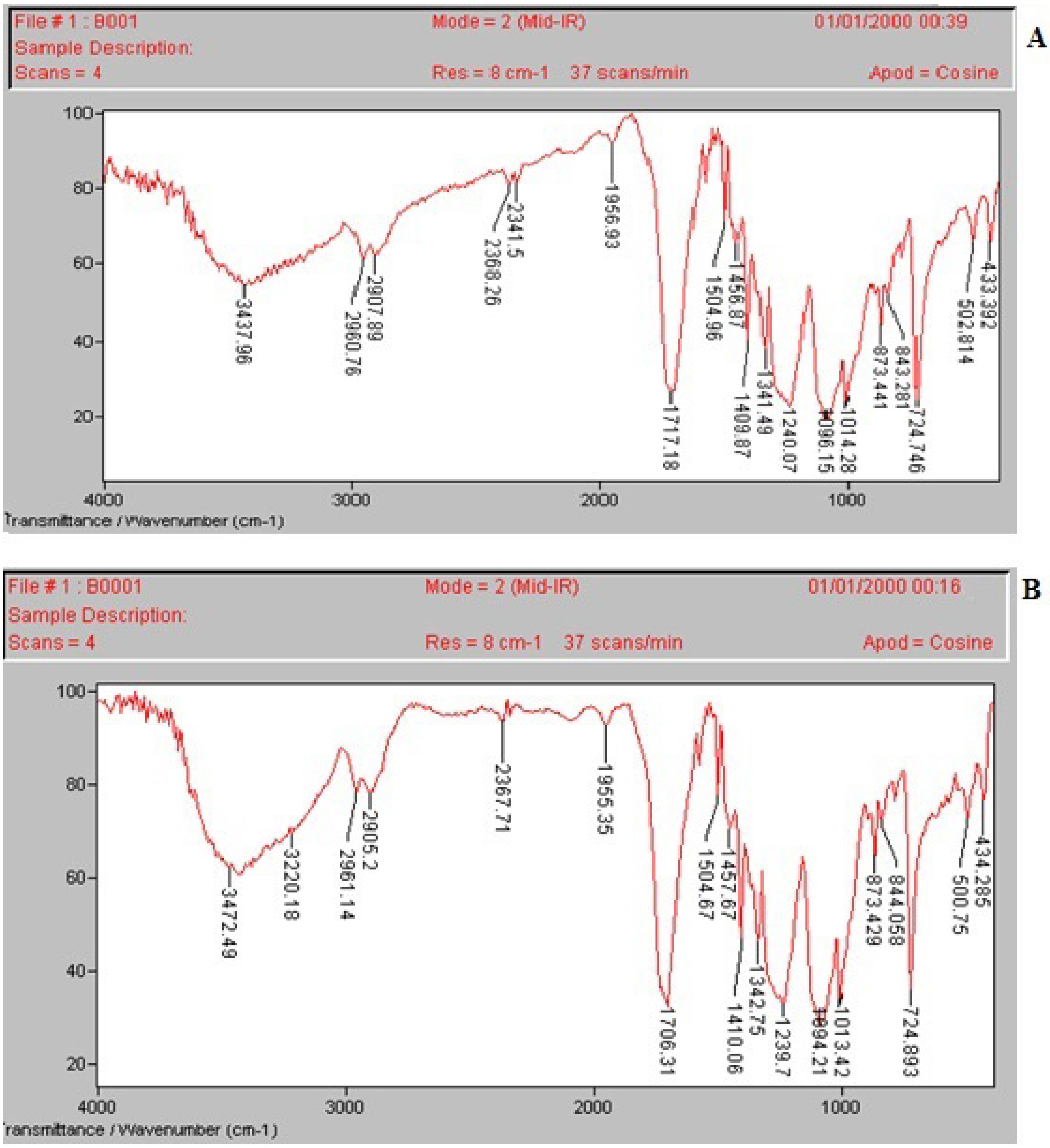

Fig. 5. FTIR analysis diagram of fabrics dyed with the violacein pigment (A). FTIR analysis of hybrid fabrics with violacein pigment and silver of titanium oxide nanoparticles (B).

After coating silver and titanium nanoparticles on the violacein pigment, the FT-IR spectra were also examined, which according to the obtained information, the synthesis of this compound was confirmed.

Due to the presence of the chemical bonds between nanoparticles and the carbonyl group, the peak of the carbonyl-amide group appeared at a lower frequency (1706 cm-1). A peak that appears at a wavelength of 1239 cm-1 is also assigned to the simple bond of oxygen and carbon. A wide peak appearing at a wavelength of 3472 cm-1 corresponds to the overlapped bonds of the tensile vibrations of O-H and N-H groups. Tensile vibrations of C-H bonds appear at wavelengths of 2905 cm-1 and 2906 cm-1 (Fig. 5B).

Violacein is a recently considered bacterial secondary metabolite. In addition to its unique therapeutic properties, it could be used as a natural dye in the textile industry.

Violacein-producing strains have been extensively studied. It has been shown that *J. lividum* strains are non-pathogenic, and this issue makes the use of this strain important in the industrial and commercial production phase. There are various methods for analyzing the antibacterial effects of dyed and hybrid fabrics, such as checking the diameter of the growth inhibition by antibiogram disks, which was not used due to the non-diffuse contents of the fabrics. So, the number of bacteria was determined in the dilution series. Previous studies have also suggested that the antibacterial activity against gram-positive strains, such as *Staphylococcus aureus* and *Bacillus* is better than that of gram-negative bacteria, such as *Escherichia coli*, but observations have shown that this may not be true and that the effect on different gram-positive strains of gram-negative bacteria is different [11]. In addition to the antimicrobial properties of the dye, the properties of this substance, in combination with other compounds, have been performed to increase its potential. For instance, silver nanoparticles coated with violacein pigments were used against some strains of *E. coli*, *Aeruginosa*, and *Staphylococcus aureus*, which was resistant to several antibiotics, and paper discs were treated with various dilutions of violacein-coated nanosilver to measure the diameter of disk diffusion [11]. These violacein-coated nanoparticles showed significant antibacterial properties. Increasing the concentration of discs containing nanoparticles increased the antibacterial activity. These violacein-coated nanoparticles not only had high antibacterial and anti-growth properties, but the values of MICs were much lower at 25 μg/ml, indicating greater activity and higher efficacy of pigments when used in association with silver nanoparticles [11, 12]. The most antibacterial effect of the designed fabrics was shown against *E. coli.* Moreover, the antibacterial activity of fabrics was more pronounced in *Pseudomonas aeruginosa* when compared with *Staphylococcus aureus*, indicating that violacein is more effective against gram-positive bacteria than gram-negative. The MIC values for violacein were 15 μg / ml for *Staphylococcus aureus*, 15 μg / ml for *Pseudomonas aeruginosa*, and less than 50 μg / ml for *E. coli*. In addition, the antifungal properties of violacein-coated nanoparticles showed that they have inhibitory effects at a range between 1-10 μg. However, the sensitivity of nanoparticles to fungi may vary due to differences in the cell wall structure of fungi. The MIC value of violacein against fungi was 5 μg / ml [17].

The EDAX analysis was performed following the abrasion and washing the hybrid fabric. The abrasion stability test and EDAX analysis showed that the stability of pigments almost remains intact. One of the most significant issues with nanoparticles is the non-diffusion and separation of these particles from the surface of the fabrics. The washing stability test is one of the necessary tests to control the quality of dyed fabrics in textiles, but because this type of fabric is only used for hospital purposes and often used as a disposable, so the analysis was not conducted. However, in case of a change in the use of this type of product, it is necessary to evaluate the quality control in terms of washing stability and to check the level of antibacterial properties. The low solubility of violacein is a major refinement in the industry. One of the proposed solutions to change the properties of this substance is its conjugation with cyclodextrins. Violacein reduces the toxicity of nanoparticles, and nanoparticles increase pigment stability. If the absorption of an antimicrobial agent, such as dye, into the fabric fibers, is low, some materials can be used to facilitate the binding process. A study has utilized a group of cross-linkers, such as glyoxal/ glycol, citric acid, and 1,2,3,4 tetracarboxylic, butane acid, to bind chitosan to cotton fabrics [21]. However, the antimicrobial activity of the designed fabrics depends on the binding of the cross-linkers to the cellulose fiber. Therefore, it is observed that the antimicrobial activity of the created fabrics is reduced by washing. However, in a method using compounds in the form of encapsulation with nanomaterials, the antimicrobial durability is improved in textile products during the washing process [1]. In one of the dyeing methods called exhaust, natural materials are used to strengthen the bond of the natural pigment on the surface of the fibers, which is performed by some materials such as alum, copper sulfate, iron sulfate, stannous chloride, tannic acid to increase the leaching resistance of natural dyes [22]. Using different compounds, the dye molecules are attached to the fabric by forming bonds. However, it has been observed that in the case of using these substances, antimicrobial activity is reduced due to the existence of a chemical bond between the hydroxyl group and other groups, and the functional groups in an organic molecule, mainly responsible for antimicrobial activity, could be affected [23]. However, some of these materials have enhanced the antibacterial properties of dyed fabrics with natural dyes, such as copper sulfate and alum. Therefore, the selection of the type of enhancers and stabilizers would be important. One study showed that the optical stability of dyes extracted from microorganisms isolated from air, soil, and water was recorded to be about 1-1.5, while such a value was about 2 for the violacein dye, suggesting a better indicator compared with previous studies. A performed on the effect of titanium dioxide hybrid nanotubes on antimicrobial activity found that the hybrid form had a greater antibacterial effect on *Escherichia coli* than *Pseudomonas aeruginosa* (both are gram-negative bacteria) [24]. This is due to the resistance of *Pseudomonas aeruginosa* to antimicrobial agents. These nano-hybrids had a greater antimicrobial effect on *Escherichia coli* than the gram-positive bacterium *Staphylococcus epidermidis*. Such resistance may be due to the cell wall structure of *Staphylococcus aur*eus, which has more peptidoglycan layers and shows better antimicrobial properties when a lower concentration of Staphylococcus epidermidis is used [25].

CONCLUSION

In general, the purpose of this study was to mass-produce a natural antibacterial dye with special properties in the industrial textile by employing biotechnological techniques and to use in hospital fabrics and textiles. In addition, this dye could also be used as a suitable alternative to chemical dyes. The current study aimed to use two types of nanoparticles with antibacterial and self-cleaning properties along with the application of violacein as a microbial dye pigment to produce a hybrid to amplify the antibacterial activity. The dyed fabrics were coated with silver and titanium dioxide nanoparticles. These fabrics were treated with nanoparticles prepared from silver and titanium dioxide, and this process was performed by sonication. The coated dye fabric was analyzed by FTIR to confirm the production pigment, formation of hybrids, and the presence of nanoparticles on the surface of fabrics. In addition, SEM images prepared from the surface of the fabric fibers confirmed the proper distribution of nanoparticles. The EDAX analysis also demonstrated that the amounts of silver and titanium dioxide nanoparticles included 1% and 36% of the mass percentage. The violacein pigment was also evident on the surface of fabrics in SEM images. In general, dyed fabrics had the desired antibacterial properties compared with control samples, and the antibacterial properties of the hybrid fabrics were enhanced. Among the three bacterial strains, *Streptococcus aureus* was significantly more sensitive than the other strains, suggesting these properties may be different depending on the type of strain.

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Declarations

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CONFLICTS OF INTEREST/COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. The biological experiments and data collection were done by Farnaz Khaksar as a MSc student in Microbial Biotechnology. The first draft of the manuscript was written by Farnaz Khaksar and all authors commented on previous versions of the manuscript. Garshasb Rigi as a supervisor of the project and corresponding author and Seyed Hossein Mirdamadian as an adviser of the study and co-corresponding author, designed the whole experiments, supported the project, analyzed data and wrote the final manuscript. All authors read and approved the final manuscript.

CONSENT FOR PUBLICATION

Not applicable

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