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Utilization of an eco-friendly bioactive yellow pigment from *Streptomyces thinghirensis* AF7 for making colored antimicrobial fabrics

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Abstract

Despite their long successful use, synthetic dyes have several problems due to their carcinogenic and toxic effects. Besides providing bright colors, some natural pigments have shown notable antimicrobial activity; thus, they could be utilized as functional dyes in many applications such as making colored antimicrobial textiles. In this work, a yellow pigment produced by *Streptomyces thinghirensis* AF7 and has a notable antimicrobial activity was used to produce a colored antimicrobial textile. The extracted yellow pigment was subjected to a purification step using silica gel column eluted with di ethyl ether solvent. The FTIR, GC-MS and NMR analysis showed that the colorings in this type of product are due to the presence of chromopeptides. The purified yellow pigment was effectively used to dye two types of fabrics (cotton and polyester). Results showed that polyester had more affinity for yellow pigment than cotton. The stability of dyed fabrics was verified based on ISO 105-E01:2013 which demonstrated that both dyed cotton and polyester fabrics had a considerable degree of fastness to water, seawater and detergent. Moreover, yellow dyed Polyester fabric exhibited more stability against acid than yellow dyed cotton fabric, and both were unstable against alkaline solution. Finally, the yellow dyed fabrics showed antibacterial properties against *S. aureus* proving that the antibacterial activities of the yellow pigment could be retained when the pigment is bound to the fabric.

Keywords: yellow pigment, *Streptomyces thinghirensis*; colored antimicrobial textiles.

استخدام صبغة صفراء نشطة بيولوجيا صديقة للبيئة من *Streptomyces thinghirensis* AF7 لصنع أقمشة ملونة مضادة للميكروبات

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الخلاصه

على الرغم من استخدامها الناجح منذ فترة طويلة، فإن الأصباغ الاصطناعية لديها العديد من المشاكل بسبب آثارها المسرطنة والسامة. إلى جانب توفير الألوان الزاهية، أظهرت بعض الأصباغ الطبيعية نشاطا ملحوظا مضادا للميكروبات. وبالتالي، يمكن استخدامها كأصباغ وظيفية في العديد من التطبيقات مثل صنع المنسوجات الملونة المضادة للميكروبات. في هذا العمل، تم استخدام صبغة صفراء تنتجها *Streptomyces thinghirensis* AF7 ولها نشاط مضاد للميكروبات ملحوظ لإنتاج نسيج ملون مضاد للميكروبات. تم إخضاع

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الصباغ الأصفر المستخرج لخطوة التنقية باستخدام عمود هلام السيليكا المنزوع بمذيب إيثيل الأثير. أظهر تحليل FTIR وMS–GC وNMR أن الألوان في هذا النوع من المنتجات ترجع إلى وجود كروموبيتيدات. تم استخدام الصبغة الصفراء النقية بشكل فعال لصبغ نوعين من الأقمشة (القطن والبوليستر). أظهرت النتائج أن البوليستر كان أكثر تقاربا مع الصباغ الأصفر من القطن. تم التحقق من استقرار الأقمشة المصبوغة استنادا إلى ISO E01: 2013–105 الذي أظهر أن كل من أقمشة القطن والبوليستر المصبوغة لديها درجة كبيرة من الثبات على الماء ومياه البحر والمنظفات. علاوة على ذلك، أظهر نسيج البوليستر المصبوغ باللون الأصفر استقرارا أكبر ضد الحمض من النسيج القطني المصبوغ باللون الأصفر، وكلاهما غير مستقر ضد المحلول القلوي. وأخيرا، أظهرت الأقمشة المصبوغة الصفراء خصائص مضادة للبكتيريا ضد *S. aureus* أثبتت أنه يمكن الاحتفاظ بالأنشطة المضادة للبكتيريا للصبغة الصفراء عندما تكون الصبغة مرتبطة بالنسيج.

Introduction

Streptomyces is a Gram-positive, filamentous bacteria belonging to the group actinomycetes [1]. They are ubiquitous in soil, conferring the characteristic earthy smell, and they have an important ecological role in the turnover of organic material [2]. Actinomycetes act as a major component of the microbial population in most soil. About 90% of the total actinomycetes population consists of *Streptomyces* species [3]. They produce a wide range of secondary metabolites; more than 70% of the naturally derived antibiotics that are currently in clinical use are derived from soil actinomycetes [4]. The genus *Streptomyces*, in particular, accounts for about 80% of the actinomycetes natural products reported to date [5]. The most interesting property of *Streptomyces* is the ability to produce bioactive secondary metabolites such as antibiotics [6].

Pigments from microorganisms, especially from *Streptomyces* strains are attractive due to the broad range of activities they have (i.e. antibiotic, antifungal, anticancer) that make them an excellent target for multifunctional applications [7]. In recent years, bio-colorant have an increasing interest due to the environmental impact resulting from synthetic dyes. As a result, increasing demand for natural pigment was observed in the environmental, food, cosmetic, textile, printing, and dye industry certainly reflected on producing these bio colorants from different natural sources such as microorganisms and plants. However, plant pigments have several disadvantages in terms of their solubility and stability in extreme pH, light, and heat that made light turn on microbial pigments which are more stable and soluble [8].

Bacteria, mold, algae, and yeast produce various pigments such as carotenoids, melanin, flavones and quinines [9]. The colored microorganisms were isolated from various samples and their extracted pigments were used successfully in different industrial applications [10]. Industrial production of natural pigments by microbial fermentation has many advantages such as cheaper production simpler extraction, higher yields through strain enhancement, no lack of raw materials, and no seasonal varieties [11].

Colors from natural sources are gaining popularity because synthetic colors are carcinogenic. Some natural colorants have shown remarkable antibacterial activity in addition to providing bright colors, which could serve as functional dyes in producing colored antimicrobial textiles. Therefore, this work was designed to obtain a bioactive pigment from a local isolate of *Streptomyces* and explore their applications in dyeing different fibers.

Material and Methods

Source of Streptomyces thinghirensis AF7

Streptomyces thinghirensis AF7 was isolated from the plant rhizosphere collected from Baghdad. This isolate produced a bioactive yellow pigment that has significant activity against different bacteria in particular *Staphylococcus aureus* and *E. coli.*

Production of bioactive yellow pigment

Yellow pigment from *Streptomyces thinghirensis* AF7 was produced in maltose casein medium was optimized for this isolate in the fermentation laboratory at the Department of Biotechnology, College of Science, University of Baghdad. This medium contained per 1 liter: 8g maltose, 5g Casein, 0.01g KNO_3, 0.05g $MgSO_4.7H_2O$, 2g K_2HPO_4, 2g NaCl, 0.02 CaCO3, 0.01 $FeSO_4.7H_2O$. pH 6. After inoculation with 5% of spore suspension, the medium was incubated at 30 ºC in a rotary shaker at 150 rpm for five days until pigment was produced into the liquid medium. After incubation, the culture broth was centrifuged at 8000 rpm for 15 min to separate the broth supernatant. Di ethyl ether (1:1) was used to separate the yellow pigment. The mixture was shaken vigorously for about 30 minutes to obtain efficient separation of crude aqueous pigment. Thereafter, the organic phase was collected by using a separation funnel and dried overnight in an incubator at 37 °C. The resulting dried pigment was then dissolved in a small amount of solvent and stored in a dark bottle [12].

Purification of yellow pigment

The extracted yellow pigment was purified using silica gel column chromatography (3 ×40) cm. Five ml of the sample was loaded gently to the column and di ethyl ether was applied for the elution. Fractions of 5 ml each were collected at a flow rate of 5 min and detected at the lambda max of the yellow pigment 449 nm until the last fraction was collected [13].

Characterization of target pigment

FTIR

FTIR spectrum of yellow pigment was detected using Shimadzu IR-470 plus in the 400 to 4000 cm^{-1} range for the solid pigment without KBr as ATR was used. The data was plotted as intensity versus wave number [14].

GC-MS

The yellow pigment was analyzed by gas chromatography-mass spectrometry (GC-MS) using a THERMO GC - TRACE ULTRA VER: 5.0, Thermo MS DSQ II with a TR 5 - MS Capillary Standard Non-Polar Column (30 m, film 0.25 μm, ID 0.25 mm). The temperature was 80 to 250°C at 8°C/min. The carrier gas was Helium with a flow rate of 1.0 mL/min. The chemical constituent was identified using NIST08.LIB library spectra are provided by the software on a GC-MS system [15].

Nuclear magnetic resonance spectroscopy (NMR)

^{1}H NMR and ^{13}C NMR were measured using Bruker 500 MHz after dissolving the sample in deuterated DMSO in the region between (0-15 ppm).

Textile dying and stability of target pigment

Textile dying and stability evaluation was achieved based on the method described by ISO 105-E01:2013. One hundred milliliters of di ethyl ether extracted yellow pigment was used to submerge two types of textiles (cotton and polyester). These textiles were carefully cut with sharp scissors to get 10×4 cm nice accurate clean edge pieces. The textile pieces were then incubated at ambient temperature for three days. The yellow pigmented textiles pieces were examined in different conditions as described in the following sections.

Colour fastness against water (ISO 105 E01)

The dyed sample was prepared by cutting 10×4 cm of the textiles pieces and putting them in a container with a flat surface and adding water until the sample was covered. The amount of distilled water exceeds a little bit more than 100 ml. Mixing the sample with a glass rod to ensure the adsorption. The dyed sample was collected and the excess solution from the fiber was removed. Thereafter, the wet fiber was pressed between acrylic plates using a press device for 4 hours at 37 ºC. The fiber was then separated from the plate and dried at 50 °C (ISO 2013).

Colour fastness against seawater (ISO 105 E02)

The dyed samples were prepared by cutting 10×4 cm of the textiles pieces and put in a container with a flat surface containing seawater (prepared by dissolving 30 g of NaCl in 1 L of distilled water) until the sample was covered. Then, the sample was mixed with a glass rod to ensure the adsorption for 30 minutes. Next, the dyed sample was collected and the excessed solution from the fiber was removed. Thereafter, the wet fiber was pressed between acrylic plates using a press device for 4 hours at 37 ºC. The fiber was then separated from the plate and dried at 50 °C (ISO 2013).

Colour fastness to perspiration (Base, Acid) (ISO 105 E04)

Acid solution

Pieces of dyed cotton and polyester fibers with 10×4 cm sizes were immersed separately in 200 ml of acid solution (prepared by dissolving 5 g of sodium chloride, 2.2 g of sodium dihydrogen phosphate, and 0.5 g of L-histidine mono-HCl mono-H2O in 1 L of distilled water) with pH 5.5. Thereafter, the immersed fiber was pressed between acrylic plates using a press device and incubated for 4 hours at 37 °C. The fiber was then separated from the plate and dried at 50 ºC (ISO 2013).

Base solution

Pieces of dyed cotton and polyester fiber with 10×4 cm sizes were immersed separately in 200 ml of base solution (prepared by dissolving 5 g of sodium chloride, 2.5 g of disodium dihydrogen phosphate, and 0.5 g of L-histidine mono-HCl mono-H2O in 1 L of distilled water) with pH 8. Thereafter, the immersed fiber was pressed between acrylic plates using a press device and incubated for 4 hours at 37 ºC. The fiber was then separated from the plate and dried at 50 °C (ISO 2013).

Colour fastness to detergent (ISO 105 C06, B2S)

Pieces of dyed cotton and polyester fiber with 10×4 cm sizes were separately immersed in 150 ml of washing solution (prepared by dissolving 1 g of sodium perborate and 4 g of CE phosphate detergent in 1 L of distilled water). Thereafter, the immersed fiber was left to stir for 40 minutes at 50 ºC. The fiber was then washed with tap water and then dried at 50 °C (ISO, 2010).

Antimicrobial activity of the dyed textiles

Dyed samples of textiles pieces were evaluated for their antimicrobial activity by disc diffusion method against Gram-positive bacteria (*Staphylococcus aureus*). 200 µL of an overnight growth culture of the tested bacteria containing approximately 1 x 10^8 cells/ml were inoculated on Muller Hinton agar. Then, dyed textile pieces were placed on the surface of the inoculated Muller Hinton agar plates. The culture plates were incubated at 37 ℃ for 24 h. After the incubation period, the plates were examined for zone of inhibition around the dyed textile pieces [16].

Results and discussion

In this work, a yellow pigment produced from the local isolate *Streptomyces thinghirensis* AF7 which has a significant antimicrobial activity was used to prepare a colored antimicrobial textile. The optimized conditions for the cultivation of *Streptomyces thinghirensis* AF7 and the production of this pigment were detected in the fermentation laboratory at the university of Baghdad (data not shown). Therefore, to obtain large quantities, the production of yellow pigment was achieved in the optimized conditions mentioned in the material and methods section. The yellow pigment was extracted from the culture broth of *S. thinghirensis* AF7 with diethyl ether as the best solvent to obtain the yellow pigment. The yellow pigment is an extracellular product; therefore, it was extracted from the cell-free supernatant of *S. thinghirensis* AF7 culture.

The extracted yellow pigment was subjected to a purification step using a silica gel column eluted with di ethyl ether solvent. Forty fractions (each of 5 ml) were collected at a flow rate of 5 min. Individual fractions collected were tested for their purity at the lambda max of the yellow pigment (449 nm). Based on the results presented in Figure (1), fractions containing the most purified yellow pigment were obtained in fractions 18 to 22 with a maximum absorbance of 0.62.

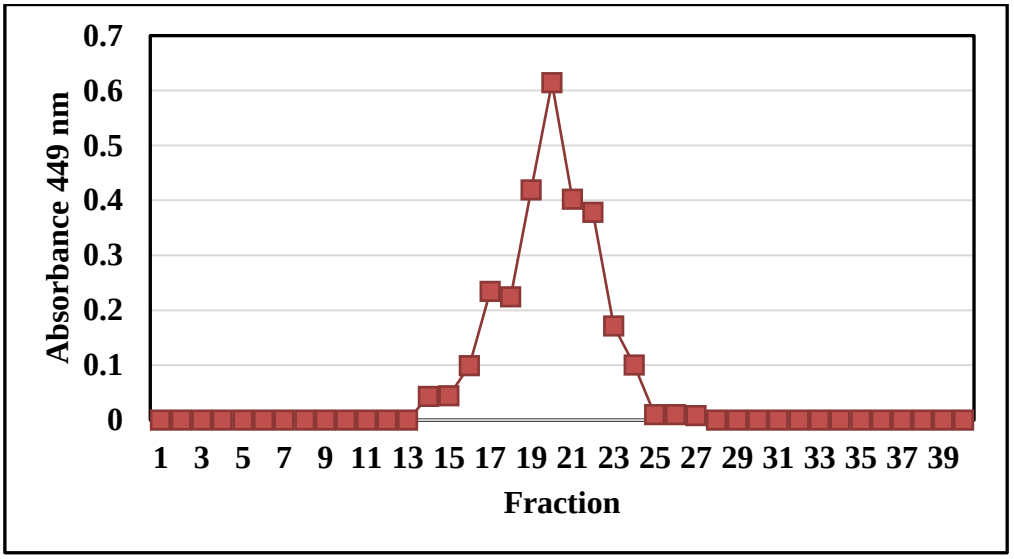


Figure 1: Purification of the extracted yellow pigment using silica gel column

Characterization of the yellow pigment

Nuclear magnetic resonance spectroscopy (NMR)

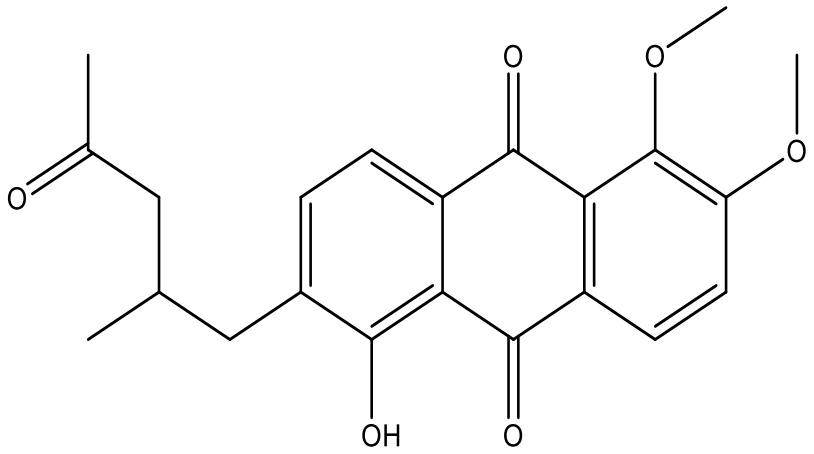
^{1}HNMR

This technique is considered as one of the most important techniques in determining what the active groups are as well as the active groups in the synthesis of organic substances. It gives conclusive evidence of the presence of the active groups and how they are related to the aromatic ring and ~~to~~ each other.

The ^{1}H-NMR spectrum (Figure 1 a and its magnified spectrum Figure 1 b) of the produced yellow pigment showed the following signals (^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.39 (d, J^3=8 Hz, 1H), 7.64 (d, J^3=8 Hz, 1H), 6.72 (d, J^3=8 Hz, 1H), 6.35 (d, J^3=8 Hz, 1H), 3.66 (s, 3H), 3.59 (s, 3H), 3.045 (d, J^3 = 8 Hz, 2H), 2.94 (d, J^3 = 8 Hz, 2H), 1.16 (m, 1H), 2.22 (s, 3H), 0.84 (d, J^3 = 8 Hz, 3H) which assigned to the following proton: CH11, CH6, CH1, CH12, CH27, CH26, CH15, CH18, CH16, CH21, and CH17, respectively [17].

The measurement proves that the yellow pigment had a very high purity, as the spectrum does not contain any additional signals, with a high separation of the signals, and therefore the compound is of high purity.

* ^{1}H-NMR= proton NMR
* DMSO-d_6= deuterated dimethyle sulfoxide solvent used in NMR spectrum
*s=singlet, d= doublet
* J=coupling constant of the proton to its neighboring proton



1-hydroxy-5,6-dimethoxy-2-(2-methyl-4-oxopentyl)anthracene-9,10-dione

Figure 2: Chemical formula of the yellow pigment (1-hydroxy-5,6-dimethoxy-2-(2-methyl-4-oxopentyl) anthracene-9,10-dione)

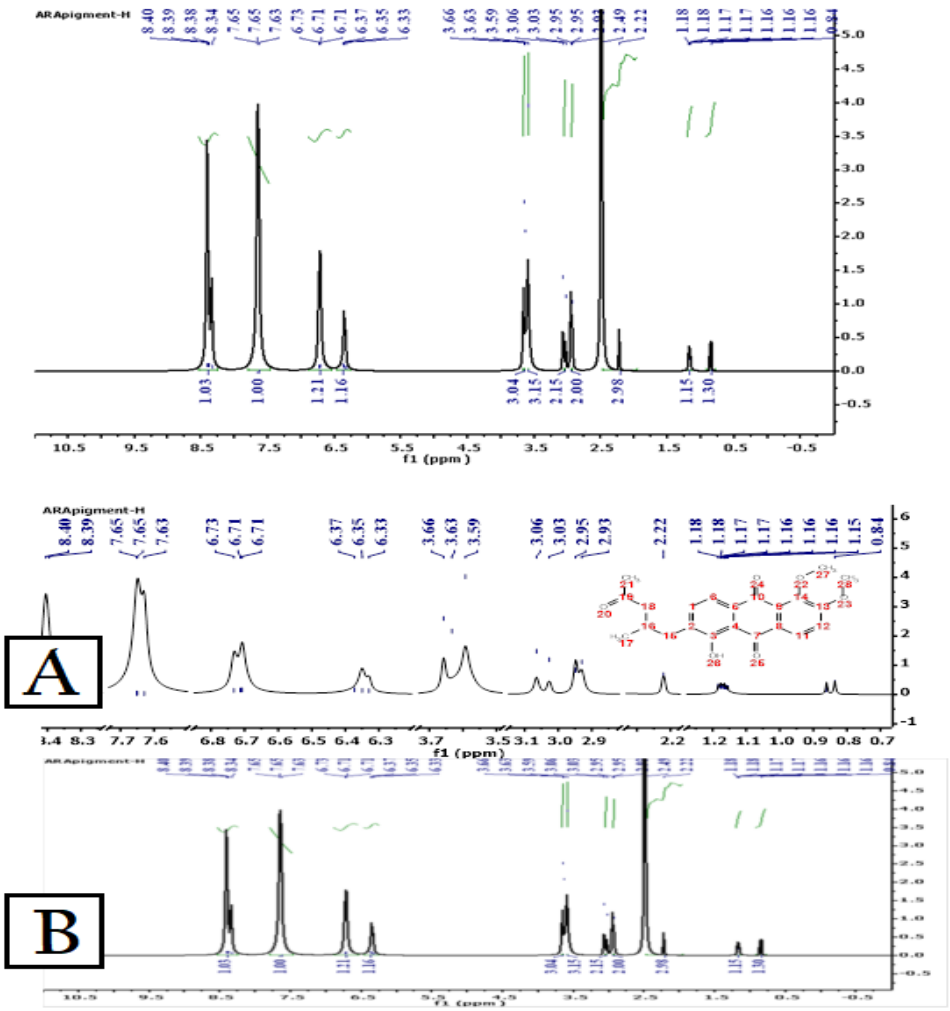


Figure 3: 1H-NMR spectrum of the isolated pigment (1-hydroxy-5,6-dimethoxy-2-(2-methyl-4-oxopentyl) anthracene-9,10-dione)

^{13}C-NMR

The ^{13}C-NMR spectrum (Figure 4) of the produced yellow pigment showed the following signals: ^{13}C-NMR (126 MHz, $CDCl_3$) δ 226.53, 189.49, 177.08, 167.20, 158.96, 151.34, 146.60, 140.52, 138.93, 129.62, 124.39, 121.57, 119.70, 114.75, 104.76, 66.95, 55.46, 48.90, 32.31, 25.93, 18.44, 9.46 which are assigned to the following carbon atoms C19, C7, C10, C3, C13, C14, C2, C5, C1, C8, C9, C6, C11, C4, C12, C27, C28, C18, C15, C16, C21 and C17, respectively. This measurement also proved the high purity of the isolated pigment [17].

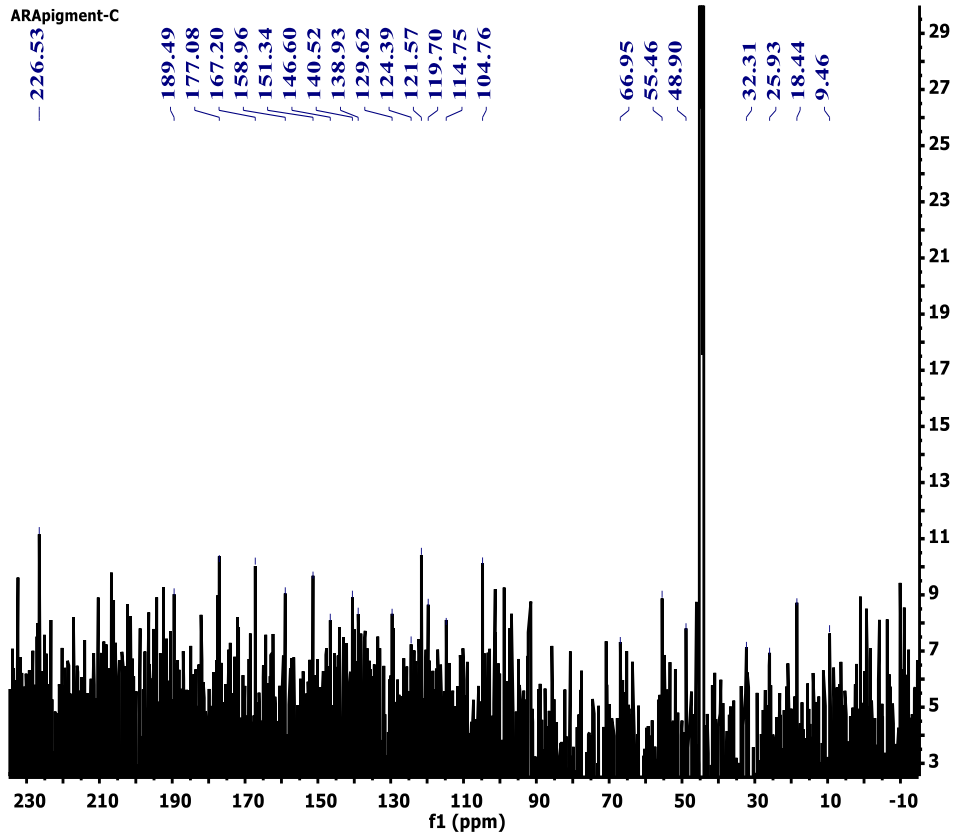


Figure 4: ^{13}C-NMR spectrum of the isolated pigment (1-hydroxy-5,6-dimethoxy-2-(2-methyl-4-oxopentyl)anthracene-9,10-dione)

Textile dying and stability of the target pigment

The purified yellow pigment was effectively used to dye two types of fabrics (cotton and polyester). Based on results obtained in this study, polyester showed more affinity for yellow pigment than cotton as mentioned in Table (1) and Figures (5,6). These findings further support the idea that synthetic fabrics had a higher affinity to natural dye compared to cotton fabrics [18]. The stability of dyed fabrics were verified based on the method described by ISO 105-E01:2013 through some tests as described in the following sections.

Colour fastness against water, seawater, and detergent

The experimental results revealed that the yellow pigment exhibited very good fastness properties (rating between 3-4) for cotton and polyester fabrics against water, seawater, and detergent Table (1). A recent study has revealed that the dyeing property of the naturally dyed cotton fabric after washing with water and different detergents was not affected [19]. It can be concluded that naturally dyed fabrics have good dyeing and wash fastness property.

Colour fastness against the acid solution

It was found that when polyester was treated with an acid solution, the pigment was retained, in contrast to cotton fabric in which the colorant was not stable in an acidic solution (Table 1). in this context, It was reported that the color of all types of Prodigiosin-dyed wool, cotton, silk, nylon and polyester textiles were stable in the acidic pH [19].

Colour fastness against a base solution

It was noted that cotton and polyester fabrics were destained when treated with a basic solution (Table 1). Mohammed and Luti (2020) have reported that the Prodigiosin-dyed textiles

including wool, cotton, silk, nylon, and polyester were reduced when treated with the alkaline solution at the basic pH [19].

Table 1: Fastness properties of dyed cotton and polyester fiber

Fiber type	Fastness properties					
	Control	Water	Seawater	Acid	Base	Detergent
Cotton	3	3	3	2	0	3
Polyester	4	4	4	4	1	4

(The result is reported according to fastness greyscale, 0 means no color, and 5 means highest dying).

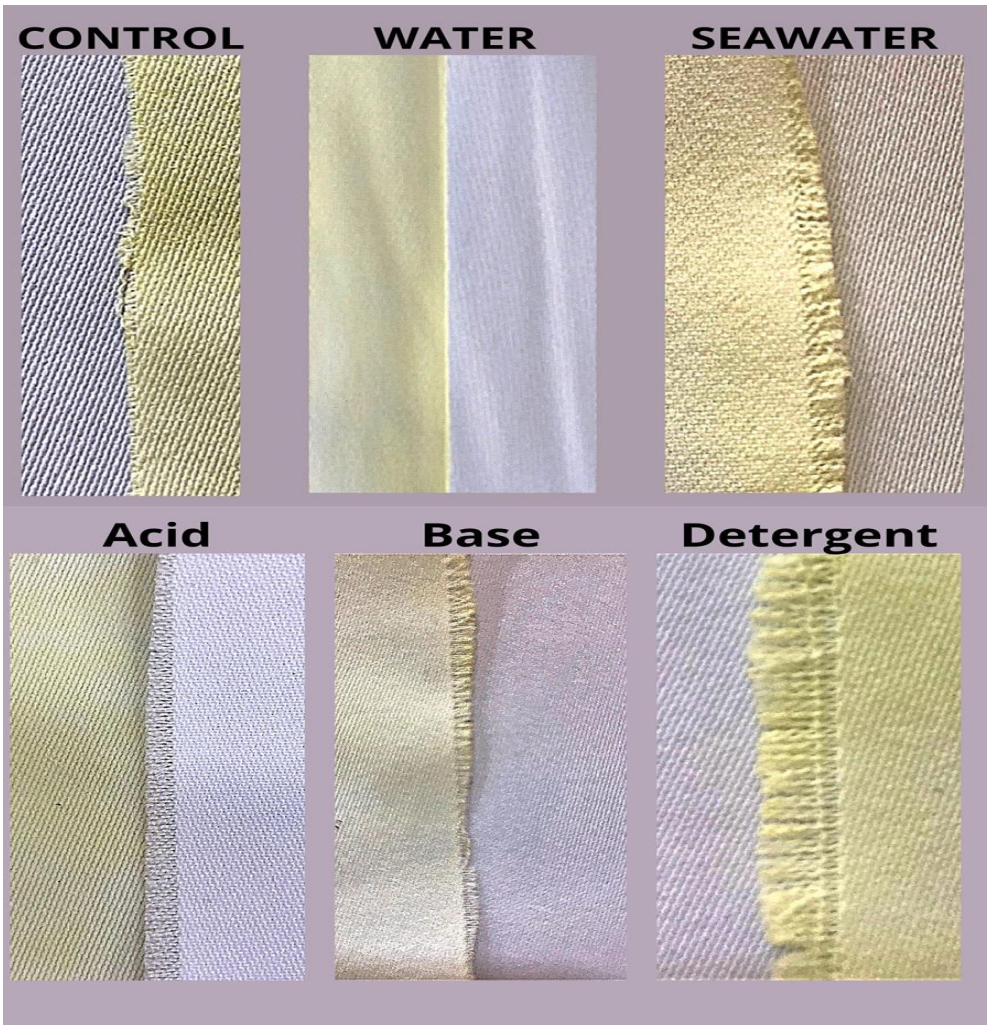


Figure 5: Fastness properties of dyed polyester fiber

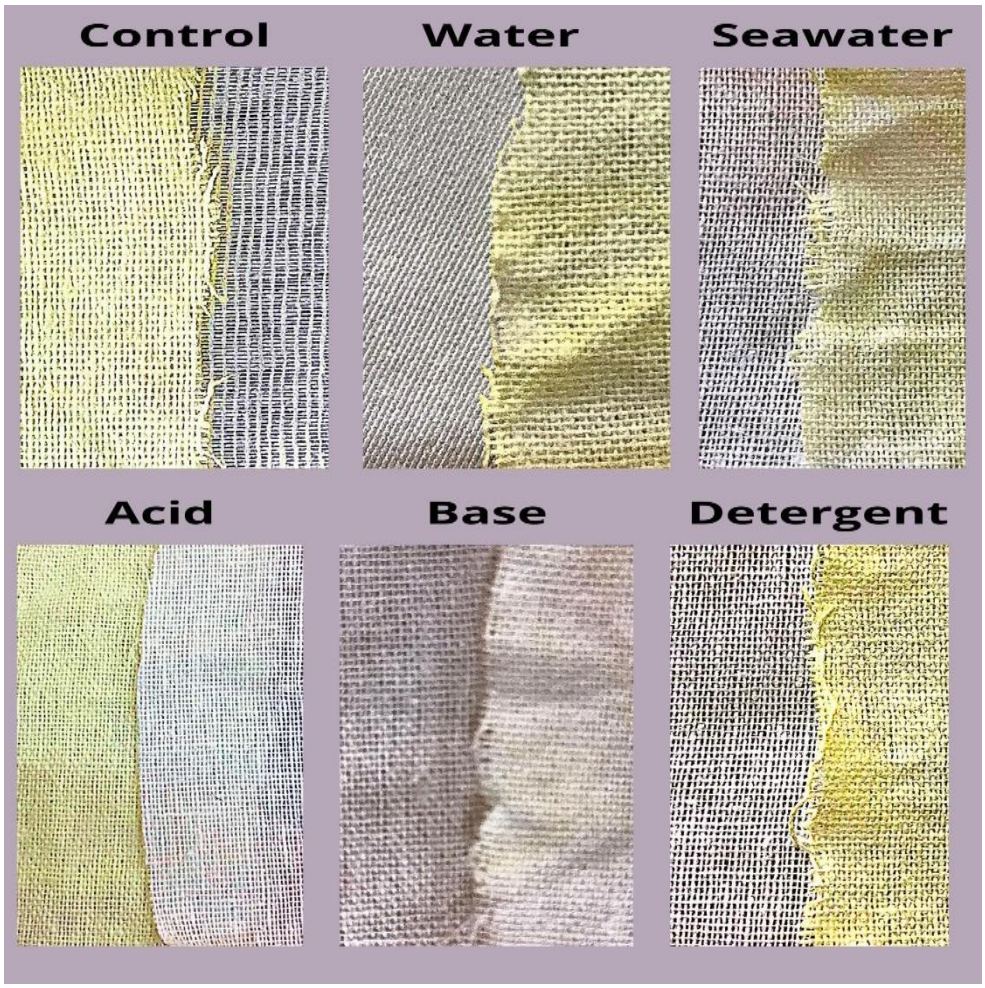


Figure 6: Fastness properties of dyed cotton fiber

The search for a new antibiotic is always active, out of all secondary metabolites having antibiotic activity, pigments are the least studied group. As the reports of pigments having antibiotic like activity are rapidly increasing, they should be studied for selective toxicity so that they can be produced commercially for human use. Natural pigments are important in the food industry as they are used as additives, color intensifiers, and antioxidants. The production of colorants from biological agents for food and textile applications has attracted increased interest in recent years, in fact, the demand for natural colorants is increasing ~~in~~ every day. Industrial production of natural colorants by microbial fermentation has several advantages such as cheaper production, easier extraction, and higher yields through strain improvement.

This study produced results that corroborate the findings of previous work in this field on using natural pigment as a colorant of textiles. One question that needs to be asked, however, is whether the antimicrobial activities of the yellow pigment that was demonstrated in this study could be retained when the yellow pigment is bound to the textiles.

Antimicrobial activity of textile materials dyed with the yellow pigment

There is an exciting challenge in using natural bacterial dyes to add antibacterial properties to textiles that are used in the medical field such as wound dressing (Gauze Pads), and to prepare garments for babies and patients who are allergic to synthetic dyes as well as used as anticancer [20].

In the current study, the yellow dyed fabrics were tested for their ability to retain antimicrobial activity. Based on the results presented in Figure (7), the yellow dyed fabrics showed antibacterial properties against infectious *S. aureus* human pathogen. These results proved that the antibacterial activities of the yellow pigment could be retained when the pigment is bound to the fabric. In this context, [20] found that the antimicrobial property of yellow pigment dyed wool sample has an excellent potentiality against *S. aureus* pathogen.

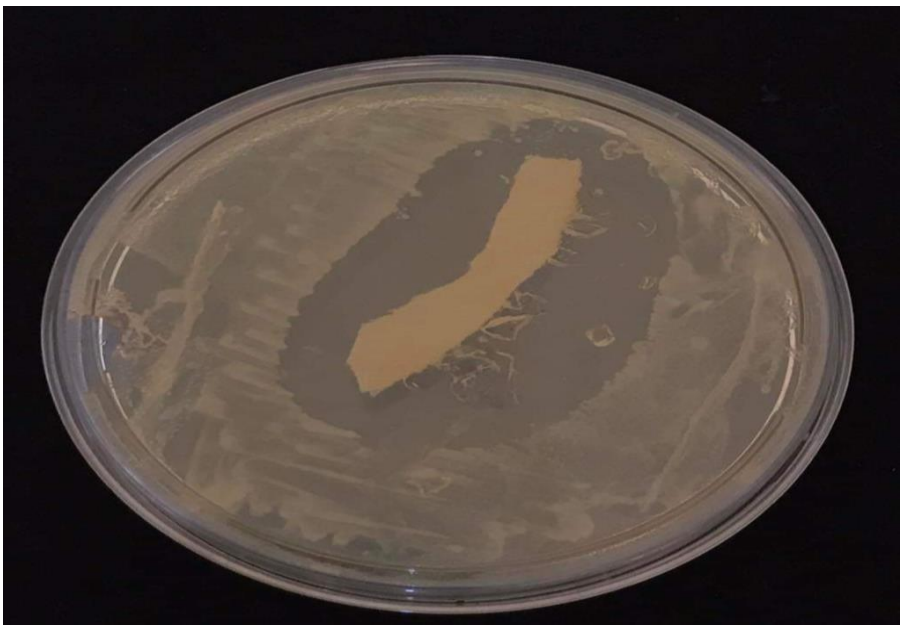


Figure 7: Antimicrobial activity of yellow dyed fabric against *S. aureus* human pathogen

Conclusions

The increasing problems due to using synthetic dyes have heightened the need for new pigments; the findings from this study make several contributions to the current literature emphasizing that microbial pigments can be a good alternative to synthetic dyes. As some microbial pigments have an antimicrobial activity which is mostly retained when the pigment is bound to the fabric, they could be used in making colored antimicrobial clothes. This study has shown that polyester had more affinity for yellow pigment than cotton. These findings further support the idea that synthetic fabrics have a higher affinity to the natural dye compared to cotton fabrics.

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