Egypt.J.Chem. **Vol. 63**, No. 6. pp. 1999 - 2008 (2020)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

A Green Approach for One Step Dyeing and Finishing of Wool Fabric with Natural Pigment Extracted from Streptomyces Thinghirensis

Tarek Abou Elmaaty[1], Ahmed Kasem[2], Mona Elsalamony[2] and Hanan Elsisi[1]
[1]*Department of Textile Dyeing & Finishing, Faculty of Applied Art, Damietta University, Damietta 34512, Egypt, Tel. and Fax: +20 572 353 098*
[2]*Department of Botany and Microbiology, Faculty of Science, Damietta University*

TODAY, natural colorants are emerging globally due to the fact that they are safer and more environmentally friendly. In this study, the dyeing substance produced by Streptomyces Thinghirensis strain (EGDA6S) was extracted by using the solvent extraction method. The extracted dyestuff was used for one step dyeing and antimicrobial finishing of wool fabrics. The dyeing properties of the dyestuff under study were investigated and the color uptake expressed as color strength was evaluated. The results revealed an excellent color uptake as well as excellent color fastness (washing, rubbing, light) that ranged (4-5). Also, the antimicrobial test showed that dyed samples have an excellent potentiality against tested pathogens.

Keywords: Streptomyces Thinghirensis, Extraction, Multifunctional dyeing, Wool.

Introduction

In last few decades, there has been an increasing destination towards replacement of synthetic dyes with natural pigments. This is a result of strict environmental standards clamped by many countries in response to the toxic and allergic reactions linked with synthetic dyes. Natural dyes are environmentally friendly; they display advantageous biological activities as antioxidants and anticancer agents. Natural dyes are exclusively obtained from plants, animals and microorganisms. Extraction of industrially important compounds from plants and animals had many blockages; it led to the loss of valuable species, was expensive and poses a great menacing in form of hazardous wastes dumped into our environment [1-4].

Microbial pigments being favored over those obtained from animals and plants. Most of the microbial pigments are safe for human use and they require less cost-effective solvents for extraction as opposed to higher plant materials [5-9]. 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 [1]. On the other hand, the antimicrobial activity of the microbial pigments looks like those obtained from antimicrobial textile finishing materials and techniques [10, 11].

Actinomycetes are microorganisms which produce various pigments on natural or synthetic media. Actinomycetes had known to produce various types of antibiotics and furthermore these antibiotics include many pigments [12-16]. Bio-pigments from microorganisms, particularly from Streptomyces strains are also catchier due to the broad ranging activities (i.e. antibiotic, antifungal, and anticancer) that make them an excellent objective for the multifunctional applications [17, 18].

This study aims to extract dyeing solution from *Streptomyces Thinghirensis* strain (EGDA6S) and dye wool fabrics with its cultural extract. In addition, the coloring and antimicrobial properties of the dyed wool fabrics would be investigated.

*Corresponding author e-mail: tasaid@du.edu.eg
Received 27/5/2019; Accepted 13/11/2019
DOI: 10.21608/ejchem.2019.13163.1829

Experimental

Materials

A 100 % Wool fabric was provided by Misr for Spinning and Weaving Company, Mahalla El-Kobra, Egypt. All chemicals used for extraction and dyeing were supplied from sigma Aldrich.

Screening and cultivation of Streptomyces Thinghirensis

The basic medium for Streptomyces culturing was starch nitrate broth and agar media. The pH was adjusted to 7, and the cultures were incubated at 30 ℃ with shaking at 150 rev min $^{-1}$ for 7 days.

The strain exhibited distinct deep yellow pigmentation on starch nitrate broth; the color was changed to orange by the time as shown in Figure 1.

Extraction and pigment yield

The crude aqueous pigment (anthracenyl methylbutenamide derivative) was collected after culture centrifugation and extracted with chloroform followed by diethyl ether. The chloroform and diethyl ether extracts were evaporated to dryness using rotary evaporator, model RV 8 S99, manufacturer IKA. The dried pigment powder was weighted.

The pigment extract in addition to the chemical structure of the dried pigment were shown in Figure 2 & Scheme 1. Nearly 0.1 g of dry pigment was obtained from 1 L of the culture.

Dyeing procedure of wool fabrics

Fabrics were premordanted by treating in a bath containing 6 % on weight of fabric (owf) with aluminum sulfate or ferrous sulfate at liquor ratio of 1: 20. Fabric was introduced into the mordant solution at 30 ℃; this temperature was maintained for 10 min and then raised to 90 ℃ over 30 min. Mordanting was continued for 60 min at this temperature. The mordant wool sample was washed with distilled water and well dried. The dye baths were prepared by dissolving the dye in 1 ml Methanol at liquor ratio 1: 20 with different dye concentrations of 2, 4 and 6 on weight of fabric (owf). Dyeing was carried out at 90 ℃ for 60 min by exhaustion method. At the end, the dyed samples were removed, soaped with 2-3 g/L aqueous solution of non-ionic detergent and PH of solution adjusted at 7.5-8 with adding sodium carbonate at 70-80 ℃ for 30 min to remove non – absorbed dyes and then dried [19]

Measurements and Testing Analysis

Color Assessment

The reflectance of the dyed samples was measured using spectrophotometer, Japan; model CM -3600, manufacturer KONICA MINOLTA. In addition to lightness (L*), Chroma (c*), hue (h*), the degree of redness (+ ve) and greenness (-ve) (a*), and the degree of yellowness (+ ve) and blueness (-ve) (b*) .The color strength (K/S) values were determined according to Kubelka – Munk equation (AATCC, 1991) as follows.

$$K/S=\frac{(1-R)^2}{2R}$$

Where "R" is the decimal fraction of the reflectance of dyed fabric. These (K/S) values represent the dye ability of the different treated and untreated samples.

Fastness Testing

The dyed samples were tested according to ISO standard methods. The tests were as follows: ISO 105-A03:1993, color fastness to washing, and ISO 105-A02:1993, color fastness to rubbing. Light fastness test was carried out after irradiation to artificial daylight for 100 hours using Phadometer light fastness tester at temperature 25 °C ± 2 °C and relative humidity 65 ± 5% alongside with a standard grey scale.

Raman Spectra of the isolated dye and wool sample.

The Raman spectra were collected using Jasco NRS-4500 in the range of 200 to 4000 cm^{-1}.

Antimicrobial activity of the extract and the dyed samples

The dried pigment was dissolved in Dimethyl Sulphoxide (DMSO) in concentrations of 20, 40, 60, 80 µg/ml. The pigment was evaluated for antimicrobial activity by disc diffusion method [20] against G +v bacteria (*Staphylococcus aureus, Bacillus cereus*, and *Enterococcus faecalis*) and G –v bacteria (*Proteus vulgaris*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia, Pseudomonas aeroginosa* and *Salmonella sp.*) in addition to *Candida albicans* (yeast). The tested microorganisms were incubated on agar plates at 37 ℃ for 24 h. After the incubation period, the plates were examined for the zone of inhibition.

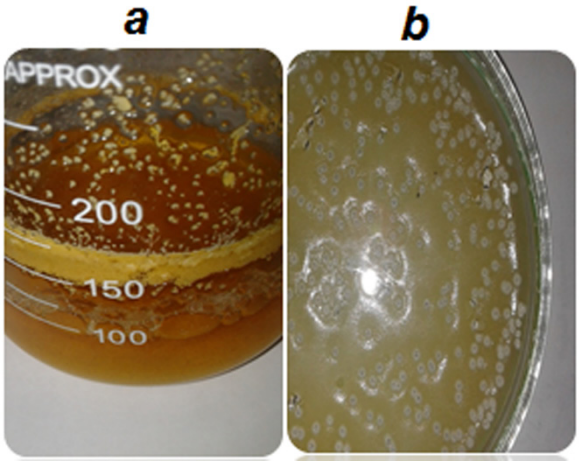


Figure 1. Growth of streptomyces thinghirensis on starch nitrate broth at 30 °C for 7 days.

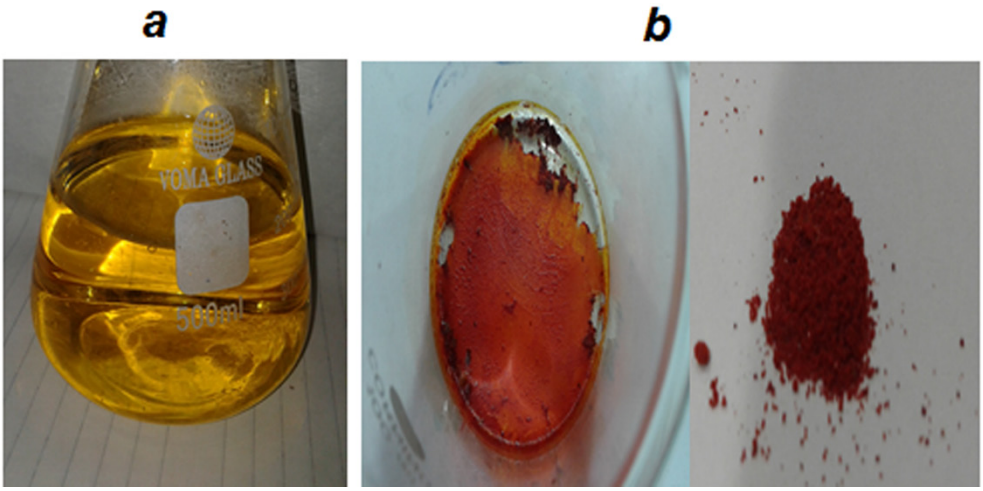
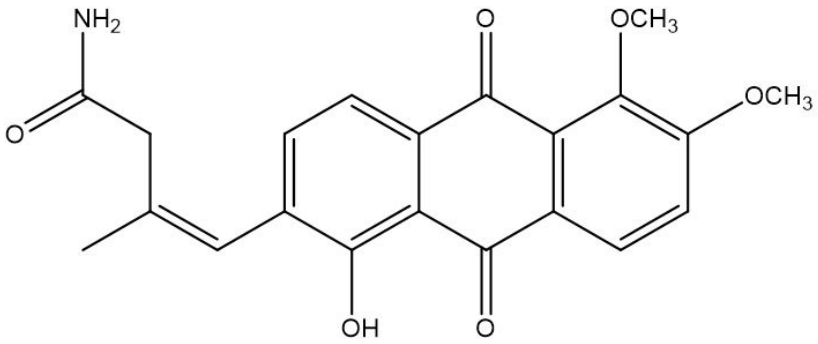


Figure 2. (a) Yellowish pigment extracted with chloroform and diethyl ether as the solvent. (b) The dry orange pigment powder after drying at 61° and 35° respectively then scratching.



4-(1-hydroxy-5,6-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-3-methylbut-3-enamide

Scheme 1. Chemical structure of pigment.

The antibacterial activity of the dyed wool sample and the pigment against G +v bacteria (*Staphylococcus aureus* and *Bacillus cereus*), G –v bacteria (*E. coli*) and *Candida albicans* (yeast) was quantitatively evaluated according to the AATCC Test Method (100 -2004) and expressed as the percent reduction of bacteria.

Results and Discussion

Dyeing properties of wool fabrics

Effect of mordant

The effect of mordant on the dye uptake, expressed as (k/s) values on wool dyeing was studied at both types of mordant alum and ferrous sulfate in addition to dyeing without mordant as shown in Figure 3. The results indicated that un-mordanted wool sample showed a lower color strength values in comparison to pre-mordanted wool fabrics. The maximum color strength obtained by using $FeSO_4$ mordant and it was higher than sample mordanted with aluminum sulphate. This may be associated with a change of ferrous sulphate into a ferric form reacting with oxygen in the air. Ferrous and ferric forms coexisted on the fibers and their spectra overlapped, resulting in a shift of λ max and consequent color change to a darker shade.

Effect of dye concentration

The dyeing properties of wool fibers were affected by many factors, such as dyeing concentration. The effect of dyeing concentration on the dye uptake expressed as color strength (k/s) of wool fabrics was investigated at a constant temperature of 90 °C, and dyeing time of 60 min. The results in Figure 4 exhibited that the color strength (K/S) values of the dyed wool samples was improved enormously with the increasing of dye concentration from 2% o.m.f. to 6% o.m.f (on the mass of the fabric). It was indicated that the dye on fiber reached saturation of adsorption with 6 %.

Color Assessment

The color of the dyed wool fabrics was evaluated using CIELAB system in terms of L^*, a^*, and b^*. The color coordinates that listed in Table 1 indicated that the dye has good affinity to wool fabrics and refers to: The dye under our study showed good affinity to wool fabrics at the given temperature and gave generally bright and deep hues ranging from yellow to orange. The color hues of the dye on fabrics were going to the yellowish direction on the yellow-blue axis according to the positive values of b^*. While going to the reddish direction on the red-green axis as indicated from the positive value of a^*.

Fastness properties

The color fastness of the dyed wool samples including washing, rubbing and light was performed according to grey scale and presented in Table 2. It is clear that, the change in color and staining by washing were excellent (rating between 4-5 and 5). The result of rubbing fastness exhibited excellent value (rating between 4-5 and 5). The result of light fastness of sample indicated very good value according to grey scale.

Raman Spectra of the isolated dye and wool sample.

Raman spectroscopy allows for the detection of dyes used in the coloration of such samples. Its potential was also demonstrated for the analysis of textile fibers. For the fiber dye analysis, Raman spectroscopy plays a complementary role that allows for identifying the main dye used for the impregnation of the fiber. The Raman technique presents advantages such as its nondestructive nature, its fast analysis time, and the possibility of performing microscopic in situ analyses. Here, spectra were collected in the range of 0 to 4000 cm^{-1}. They were acquired using scan time settings of 50 s and a resolution of 2.32 cm^{-1}/ pixel for fiber analysis. A comparison of Raman spectra for dyed and undyed fabrics was given. Figure 5 showed Raman spectra of the undyed and dyed samples with isolated crystal pigment of wool fabrics where the waves assigned as follows: 1560 (C=C aromatic), 1660 (C=O), (NH_2), 1635 (C=O), 3660 (NH_2), 3015 (CH unsaturated). The recorded Raman spectra unequivocally confirmed that all the designed and expected characteristic groups were involved in the chemical structures of the obtained dyes.

Antimicrobial activity of the extract and the dyed sample

Antimicrobial activity of the extracted pigment illustrated in Table 3 against some G +v and G –v bacteria in addition to *Candida*. The present results indicated that the pigment is highly effective against *Candida albicans*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Bacillus cereus* as shown in Figure 6. The extracted pigment showed a little activity against *Proteus mirabilis*

(With dye concentration of 2% on weight of fabric (owf) at 90 ℃ for 60 min).

TABLE 1. Color coordinates and K/S values of the dyed wool samples.

Type of mordant	L*	a*	b*	C*	h*	K/S
Without mordant	75.3	11.4	52.9	54.6	78.5	3.6
Alum.	77.4	11.7	55.1	56.3	78.1	3.9
Ferrous sulfate	62.1	12.4	47.6	49.3	75.3	7.5

(With dye concentration of 6% on weight of fabric (owf) at 90 ℃ for 60 min).

TABLE 2. Fastness properties of dyed wool fabric. (Units from 0-5, 0 is the lowest and 5 is the highest).

Fastness properties of dyed wool				
Wash fastness		Rubbing fastness		Light fastness
Color change	Staining	wet	dry	
4-5	4-5	4	4-5	4

TABLE 3. Antimicrobial activity of the pigment at different concentrations at 37 ℃ for 24 h.

Test organism	Zone of inhibition in (mm)				
	control	20 µg/ml	40 µg/ml	60µg/ml	80µg/ml
Enterococcus faecalis	-	17	19	20	22
Staphylococcus aureus	-	10	14	16	19
Bacillus cereus	-	14	19	20	22
Proteus vulgaris	-	16	17	19	22
Escherichia coli	7	10	12	20	22
Proteus mirabilis	-	-	-	7	8
Klebsiella pneumonia	-	-	-	-	-
Pseudomonas aeroginosa	-	-	-	-	-
Salmonella sp.	-	-	-	-	-
Candida albicans	-	16	18	19	20

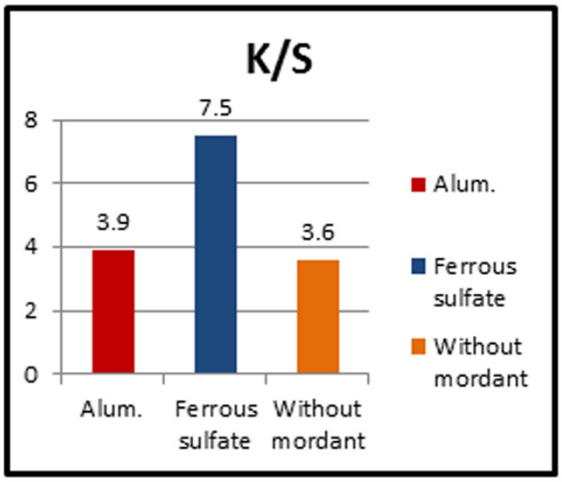


Figure 3. Effect of mordanting on K/S values of dyed wool samples (with dye concentration of 2% on weight of fabric (owf) at 90 ℃ for 60 min).

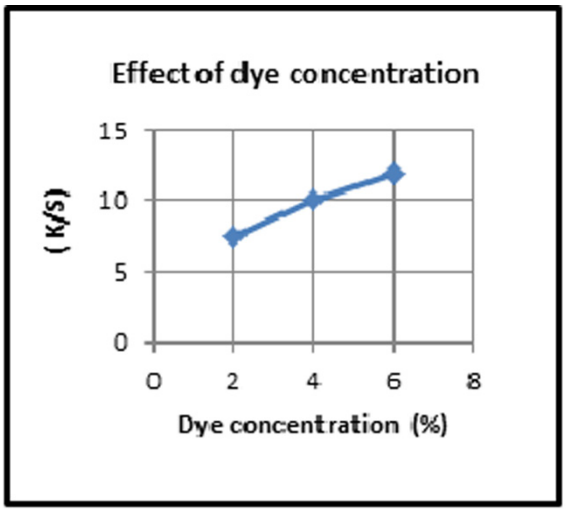


Figure 4. Effect of dye concentration on K/S values of dyed wool samples at 90 ℃ for 60 min.

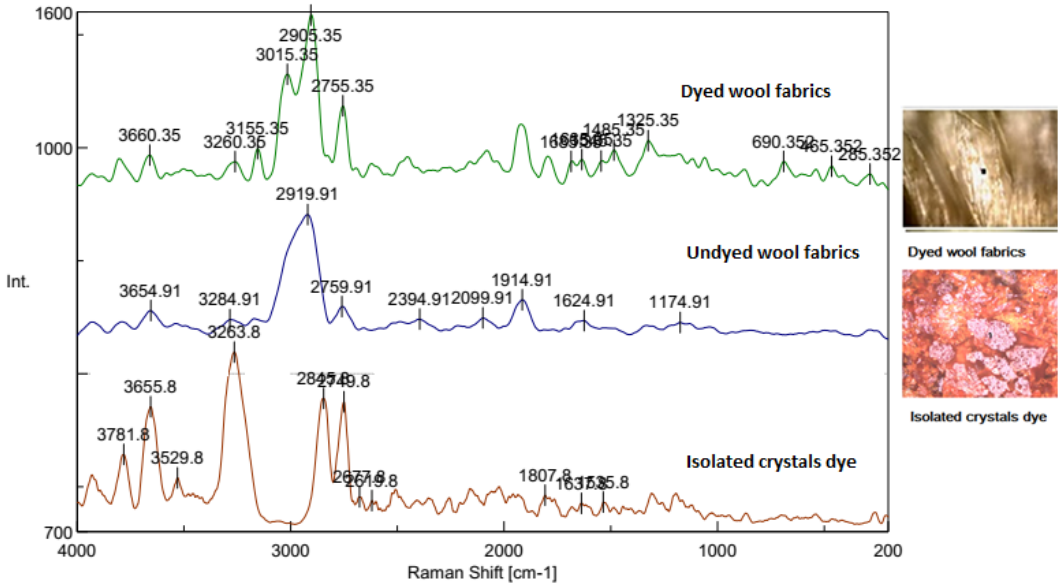


Figure 5. Raman spectra of isolated crystal pigment and undyed and dyed wool fabrics (with dye concentration of 6% on weight of fabric (owf) at 90 ℃ for 60 min).

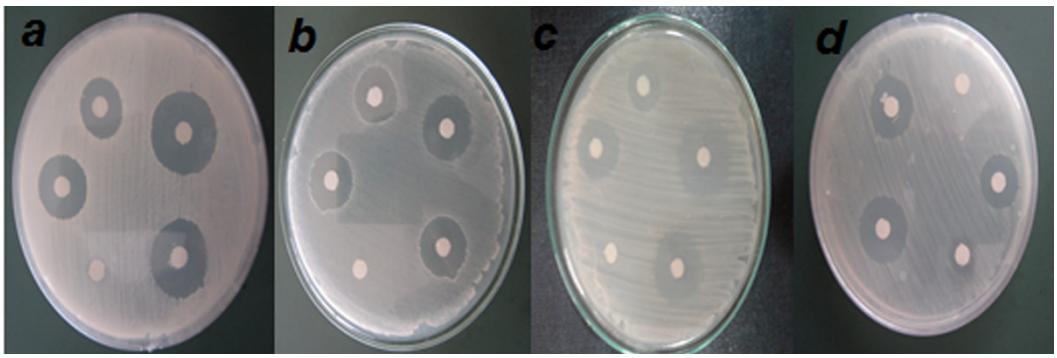


Figure 6. Antimicrobial activity of pigment against (a) *Proteus vulgaris* (b) *Candida albicans* (c) *Bacillus cereus* (d) *Staphylococcus aureus* at 37 ℃ for 24 h.

and no action against *Klebsiella pneumonia, Pseudomonas aeroginosa* and *Salmonella sp*. It was observed that the increasing in pigment concentration leads to increased inhibition reflected by enhancement in diameter. It may be concluded that the pigment is highly effective antimicrobial agent as its inhibitory concentration lies in region 20-80 µg/ml. The obtained results were found to be in agreement with previously reported [21] research which proved the potentiality of *Streptomyces Thinghirensis* as an antimicrobial agent against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*. Furthermore, *Streptomyces lienomycini* produced also a yellow pigmentation which exhibited considerable antimicrobial activity towards the same pathogenic bacteria [22]. The extract of yellow pigment produced by *Streptomyces hygroscopicus* showed also antimicrobial effect for some tested bacteria [23].

The antimicrobial activity of the dyed wool sample and the pigment against *Staphylococcus aureus*, *E. coli, Candida albicans* and *Bacillus cereus* was assessed. Tables 4 and 5 showed the antimicrobial results of the dyed wool sample and the pigment. The antimicrobial results revealed that the dyed fabric and the pigment had approximately similar antimicrobial activity against *Staphylococcus aureus*, *E. coli, Candida albicans* and *Bacillus cereus.*

TABLE 4. Antibacterial activity of the pigment using reduction test method.

Samples	*Staphylococcus aureus*	*E. Coli*	*Candida Albicans*	*Bacillus cereus*
control	0 %	0 %	0 %	0 %
20 µg/ ml	98.23%	99.98%	98.02%	99.93 %

TABLE 5. Antibacterial activity of the dyed fabric using reduction test method.

Samples	*Staphylococcus aureus*	*E. Coli*	*Candida Albicans*	*Bacillus cereus*
Control	0 %	0 %	0 %	0 %
Wool	96.79 %	99.30 %	97.96 %	98.89%

Conclusion

Our study showed that isolated pigment (anthracenyl methylbutenamide derivative) from the Streptomyces Thinghirensis can be successfully used for dyeing wool fabrics under optimized conditions of dye concentration of 6% (owf) at 90 for 60 min to obtain a wide range of soft and light colors by using different types of mordant on wool dyeing. Sample mordanted with ferrous sulphate exhibited the highest k/s values. The color fastness of dyed wool samples showed excellent results. The pigment under study showed significant antimicrobial activity against tested Gram positive such as *Enterococcus faecalis*, Gram negative bacterial pathogens such as *Proteus vulgaris* and pathogenic yeast of genus *Candida*. The obtained results qualify the treated fabric for possible medical application.

Conflict of interest

The authors declare no conflict of interest.

References

1. Malik K., Tokkas J., and Goyal S., Microbial pigments: a review. *International Journal of Microbial Resource Technology* 1, 361-365(2012).

2. Venil C. K., Zakaria Z. A., and Ahmad W. A., Bacterial pigments and their applications. *Process Biochemistry* 48, 1065-1079 (2013).

3. Nagia F. and El-Mohamedy R., Dyeing of wool with natural anthraquinone dyes from Fusarium oxysporum. *Dyes and Pigments* 75, 550-555 (2007).

4. Elnagar K., Abou Elmaaty T., and Raouf S., Dyeing of polyester and polyamide synthetic fabrics with natural dyes using ecofriendly technique. *Journal of Textiles*. 2014, 8 (2014).

5. Palanichamy V., Hundet A., Mitra B., and Reddy N., Optimization of cultivation parameters for growth and pigment production by Streptomyces spp. isolated from marine sediment and rhizosphere soil. *International Journal of Plant, Animal and Environmental Sciences* 1, 158-170 (2011).

6. Perumal K., Stalin V., Chandrasekarenthiran S., Sumathi E., and Saravanakumar A., Extraction and characterization of pigment from Sclerotinia sp. and its use in dyeing cotton. *Textile research journal* 79: 1178-1187 (2009).

7. Elmaaty T. A., Abdelaziz E., Nasser D., Abdelfattah K., Elkadi S., and El-Nagar K., Microwave and nanotechnology advanced solutions to improve

ecofriendly cotton's coloration and performance properties. *Egyptian Journal of Chemistry* 61, 493-502 (2018).

8. Ramadan M. A., Nassar S.H., Abd El Aty A., Nassar M., Elshamy A., Montaser A., and Kantouch F., Antimicrobial fabrics using Conocarpus erectus aqueous extract. *Egyptian Journal of Chemistry* 60, 1111-1121 (2017).

9. Helmy H., Kamel M., Hagag K., El-Hawary N., and N. S. El-Shemy N., Antimicrobial activity of dyed wool fabrics with peanut red skin extract using different heating techniques. *Egyptian Journal of Chemistry* 60, 103 - 116 (2017).

10. Ibrahim N., Eid B., Abou Elmaaty T., and El-Aziz E., A smart approach to add antibacterial functionality to cellulosic pigment prints. *Carbohydrate polymers* 94, 612-618(2013).

11. Elmaaty T. A., El-Nagare K., Raouf S., Abdelfattah K., El-Kadi S., and Abdelaziz E., One-step green approach for functional printing and finishing of textiles using silver and gold NPs. *RSC Advances* 8, 25546-25557(2018).

12. Amal A. M., Abeer K. A., Helmy S., and Abd El-Nasser N., Selection of Pigment (Melanin) production in Streptomyces and their application in Printing and Dyeing of Wool Fabrics. *Research Journal of Chemical Sciences* 1, 22-28 (2011).

13. Azimi S., M. Basei Salehi M., and Bahador N., Isolation and Identification of Streptomyces ramulosus from Soil and Determination of Antimicrobial Property of its Pigment. *Modern Medical Laboratory Journal* 1, 36-41(2017).

14. Parmar R. S., C. Singh C., Saini P., and Kumar A., Isolation and screening of antimicrobial and extracellular pigment producing actinomycetes from chambal territory of Madhya pradesh region, India. *Asian Journal of Pharmaceutical and Clinical Research* 9, 157-160 (2016).

15. Arasu M. V., Duraipandiyan V., Agastian P., S. Ignacimuthu S., Antimicrobial activity of Streptomyces spp. ERI-26 recovered from Western Ghats of Tamil Nadu. *Journal de Mycologie Médicale* 18, 147-153 (2008).

16. Sathi Z. S., Sugimoto N., Khalil M. L., and Gafur M. A., Isolation of yellowish antibiotic pigment 4-hydroxy nitrobenzene from a strain of Streptomyces. *Pakistan Journal of Biological Sciences* 52, 201-203(2002).

17. Babitha S, Microbial Pigments. In: Singh nee' Nigam P., Pandey A. (eds) Biotechnology for Agro-Industrial Residues Utilisation. Springer, Dordrecht 147-162(2009).

18. Dufossé L, Pigments, Microbial. M. Schaechter (Eds), Encyclopedia of Microbiology, Elsevier Publisher 457-471(2009).

19. Ghaheh F. S., Mortazavi S. M., Alihosseini F., Fassihi A., Nateri A. S., and Abed D., Assessment of antibacterial activity of wool fabrics dyed with natural dyes. *Journal of Cleaner Production* 72, 139-145 (2014).

20. Dekić B. R., Radulović N. S., Synthesis and antimicrobial activity of new 4-heteroarylamino coumarin derivatives containing nitrogen and sulfur as heteroatoms. *Molecules* 15, 2246-2256 (2010).

21. Hasaneen M. N., El-Sayed A. K., Sabry S. M., *Agriculture Chemistry and Biotechnology* 9, 263-268 (2018).

22. El-Naggar N. and Hamouda R., Antimicrobial Potentialities of Streptomyces lienomycini NEAE-31 against Human Pathogen Multidrug-resistant Pseudomonas aeruginosa. *International Journal of Pharmacology* 12, 769-788 (2016).

23. Selvameenal L., Radhakrishnan M., and Balagurunathan R., Antibiotic pigment from desert soil actinomycetes; biological activity, purification and chemical screening. *Indian journal of pharmaceutical sciences* 71, 499-504 (2009).

نهج اخضر لصباغه وتجهيز الاقمشه الصوفيه بملونات طبيعيه مستخرجه من ستربتوميسيس ثنجرنسيس

طارق ابوالمعاطي[1]، احمد قاسم[2]، مني السلاموني[2]، حنان السيسي[1]

[1]قسم طباعه المنسوجات والصباغه والتجهيز، كليه الفنون التطبيقيه، جامعه دمياط

[2]قسم النبات، كليه العلوم، جامعه دمياط

يهدف هذا البحث إلى انتاج صبغة من السلالة Streptomyces thinghirensis والتي تم استخلاصها باستخدام كلا من الكلوروفورم و ثنائي إيثيل الإيثركمذيبات عضوية. تم دراسة ومعرفة مدى قابلية استخدام المستخلص كمادة مضادة لنمو البكتيريا وبالتالي يتم استخدامه في صباغة أقمشة مقاومة للبكتيريا. أوضحت النتائج أن هذا المستخلص مضاد لنمو بعض البكتيريا الممرضة بالإضافة الى الكانديدا .تم استخدام هذا المستخلص في صباغة أقمشة الصوف، الي جانب دراسة مدى تأثيره على خواص الثبات للأقمشة وهي الثبات للغسيل، الثبات للضوء، الثبات للاحتكاك وقوة اللون والتي أظهرت نتائج جيدة. وتم عمل اختبار مقاومة نمو البكتيريا للأقمشة المصبوغة وأظهرت النتائج أن الأقمشة لها مقاومة جيدة لنمو بعض البكتريا الممرضة بالإضافة الى الكانديدا.