EXPLORING THE POTENTIAL OF PRODIGIOSIN AS AN ANTIMICROBIAL AGENT IN TEXTILES

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Abstract: Dyes and pigments are used by humans to add colors in their life. Due to the hazardous impact of synthetic azo dyes on the environment and also on human health, there is an increasing demand for natural dyes in the food, cosmetics, pharmaceutical, and textile industry. Natural dyes of biological origin have been extracted from plants, algae, and various pigment-producing microorganisms. Microorganisms are fast-growing and it produces a large amount of the pigment using cheap carbon and energy sources for their growth. It is easy and inexpensive to extract microbial pigments than pigments from plant sources. Also, microbial pigments are biodegradable and have no negative impact on the environment.

Prodigiosin is a family of tripyrrole ring-containing natural red pigment produced as a secondary metabolite by the Gram-negative bacteria, *Serratia marcescens* which is reported to have antibacterial, antifungal and anti-proliferative activity. In the present study, prodigiosin pigment was produced and extracted from an already characterized *Serratia marcescens sp.* using organic solvent. The extracted pigment was used to dye cotton and synthetic fabrics. Antibacterial activity of dyed fabrics against skin infections causing pathogenic bacteria was assessed by the agar diffusion method. The study reveals that the dyed fabrics have antimicrobial activity against the pathogenic test organisms. Thus it can be concluded that apart from imparting bright colors, prodigiosin also imparts anti-microbial finish to the textile fabrics.

Keywords: *Antimicrobial activity, Microbial dye, Prodigiosin, Serratia.*

I.INTRODUCTION

Pigments or colors are classified based on the source as natural or synthetic. Natural pigments are produced by living organisms such as plants, animals, and microorganisms. Synthetic pigments are compounds that are synthesized by chemical methods. Natural organic pigments have a significant role in food, cosmetics, dyeing textile etc. Natural organic pigments of biological origin are usually extracted from various plant parts and from certain pigment-producing microorganisms (Aberoumand, 2011). Although, since ancient times, natural pigments are used to impart colors to clothes, paper, and paints etc., synthetic dyes have replaced the natural dyes in present times because they are easily available as well as cost-effective (Saxena, 2014).

The textile industry is one of the rapidly growing industries worldwide, which utilizes enormous amounts of synthetic dyes. In recent times there is an escalation in the use of synthetic dyes in textile dyeing industry. Untreated effluents rich in color and residues of reactive dyes and chemicals from these industries are being released onto the water bodies which eventually poison, damage or affect one or more species in the environment, with resultant changes in the ecological balance (Khan, 2014).This has now become a serious concern for environmental pollution. Synthetic food colors commonly used in food industry are known to cause hypersensitivity reactions in humans and are carcinogenic in nature (Amchova, 2015). With the increasing concern for health, people avoid using synthetic colors and desire natural food colorings which can be nutritious too (He, 2010).

Although there are a number of natural pigments, only a few are available in sufficient quantities to be useful for industrial purposes (Venil, 2013). As a consequence there is a revived interest in the search for good sources of natural pigments, which are eco-friendly and biodegradable in the environment and non-toxic to human health. There is an ever-increasing interest in microbial pigments due to several reasons like less production time giving high yield, safety of the pigment which has no negative impact on the environment, and compared to plant sources, the production is flexible and can easily be controlled. A large number of different species of bacteria, molds, yeasts and algae are used to produce pigments.

Prodigiosins are a family of naturally occurring tripyrrole ring-containing red pigments (Krishna, 2008) produced by bacteria like *Serratia marcescens, Serratia rubidaea, Alteromonas rubra, Vibrio gazogenes*, etc. This pigment has distinct antibacterial, antimycotic, immunomodulating (Vijayarani, 2013), anti-tumor, anti-malarial (Patil, 2011) and anti-cancer activities (PB, 2019)

The main objective of the study is to produce and extract prodigiosin pigment from the isolate *Serratia marcescens.* The pigment will be evaluated for its application as a dye in coloring various textile fabrics using a different mordant. The stability and wash performance of the dyed fabrics in detergent will be determined. Further, the antimicrobial activities of the dyed fabrics against skin infection-causing microbes will be evaluated. The fabrics having antimicrobial activities can be further studied for their applications as wound dressings, hospital beddings etc.

II. MATERIALS AND METHODS

2.1 Optimization, lab-scale production, and extraction of the pigment

A characterized strain of *Serratia marcescens* which was isolated in our lab was selected for the prodigiosin production. Prior to the cultivation of bacteria for dye production, the growth conditions which favored pigment production, like type of medium (nutrient broth or nutrient agar), media (nutrient broth, nutrient broth + 1% to 5% glycerol, nutrient broth + 1% to 5%

mannitol, pH of medium (2 to 12), incubation temperature (20 °C to 60 °C), incubation time (24 hr to 96 hr) were optimized. Optimization of production parameters was performed by considering one factor at a time.

Production of prodigiosin was carried out in 200 ml Nutrient broth containing 1% mannitol and 1% glycerol. Flask was incubated at 37°C for 24 hrs on a rotary shaker. After incubation the broth was centrifuged at 10000 rpm for 15min and supernatant was discarded. Pellet was dissolved in 10 ml of methanol. Further pellet suspension was sonicated at 50Hz for 15 min. The sonicated solution was centrifuged at 12,000 rpm for 10 min. Pellet was discarded while supernatant was concentrated by evaporation at 60°C for 3 h in hot air oven. The extract was dissolved in 95% methanol and stored below 10 °C.

2.2 Characterization of prodigiosin

The red pigment in methanol was taken in two test tubes. The contents of one of the test tubes were acidified with a drop of conc. HCl and the other alkalinized with a drop of conc.NH_3solution. The development of pink color in the acidified solution and yellow color in the alkaline solution indicates a positive test for prodigiosin (Gerber, 1976). The red pigment was tested for stability at various pH ranging from pH 2 to 12 by using acetate buffer and borate buffer. Methanol used as control was colorless at all the pH range.

The colored solution was then analyzed by measuring the absorbance in a UV-Visible spectrophotometer for detecting the λ_{max}. The scanning range selected was 400-700 nm.

2.3 Purification of the pigment

The extracted pigment was purified and separated by thin-layer chromatography (TLC). The solvent system used for the TLC was chloroform: methanol: acetic acid: water (90: 8:1.2:0.8). After 45 min, the TLC sheet was carefully removed and the Retention factor (Rf) value was calculated according to the following equation from the chromatogram.

Rf= Distance travelled by the pigment /Distance travelled by the solvent front

2.4 Dyeing different textile fabrics

Different types of commercially available textile fabric samples like cotton, terry-cotton, wool, and satin were selected for the experiment. Each material was cut into equal size of 2 cm x 2 cm squares. The bacterial pigment in methanol was used as the stock solution. From this stock solution 500µl was applied to the cloth material. The dyed fabric was allowed to dry at room temperature for about 1 h. One set of experiments was done with the application of, as a mordant. The dyed cloth materials were dipped in mordant solution, (l %) Thiourea and (l %) Tartaric acid for 30 min at 60°C. For all the experiments white cloth fabric was taken as a control.

2.5 Wash performance of the dyed fabrics

All the dyed textile materials were dipped in an l % detergent solution and kept for 30 min at 40 ^{0}C. After 30 min the fabrics were washed with tap water and allowed to dry at room temperature (28 ± 2°C). The absorbance of the solution after washing was measured at 535 nm in a UV-Visible spectrophotometer. Distilled water was used as blank for the experiment. The same procedure was repeated for the dyed textile material treated with thiourea and tartarate.

2.6 Antimicrobial activity of the dyed fabrics

Standard cultures of skin infection-causing pathogens like *Staphylococcus aureus* MTCC3160 (Cohen, 2007), Pseudomonas *aeruginosa* MTCC 1688 (Siebenhaar, 2007) and Candida albicans MTCC 3017 (Dąbrowska, et al., 2019) were swabbed onto sterile Mueller-Hinton agar and dyed fabrics were placed on the agar surface. The plates were incubated at 37 ºC for 24 hr. The inhibition zone diameter was measured in mm. The white fabric was used as a control.

III. RESULTS AND DISCUSSION

3.1 Optimization of production conditions and production of prodigiosin

The optimization of the production conditions was performed before production was carried out. *Serratia marcescens* gave a maximum yield of pigment at temperature 37°C (Fig. 1a), pH 7 (Fig. 1b), after incubation for 24hr (Fig. 1c), in nutrient broth medium with 1%mannitol and 1% glycerol. Nutrient broth medium with both glycerol and mannitol produced more yield as shown in (Fig.1d). An increase in the concentration of glycerol and mannitol more than 1% did not produce a significant increase in the yield of the pigment. Prodigiosin production was carried out as per optimized conditions.

3.2 Extraction and characterization of the pigment

The pigment was extracted using methanol. Pigment on acidification with a drop of conc. HCl developed dark red color and on alkalinization with a drop of conc.NH_3 solution developed a pale yellow color (fig. 2).

In an acid medium (pH 2), the pigment appears red and exhibits a sharp spectral peak at 535 nm. In an alkaline medium (pH 10), the pigment is colored orange-yellow and possesses a broader spectral curve centered at 470 nm and in neutral medium (pH 7) the λ_{max} was found at 540nm (Fig. 3) (Rakh, 2017)

3.3 Purification of the pigment

The extracted pigment was separated by thin-layer chromatography (TLC). After 45 min, single spot with Rf value 0.48 was obtained (Fig.4).

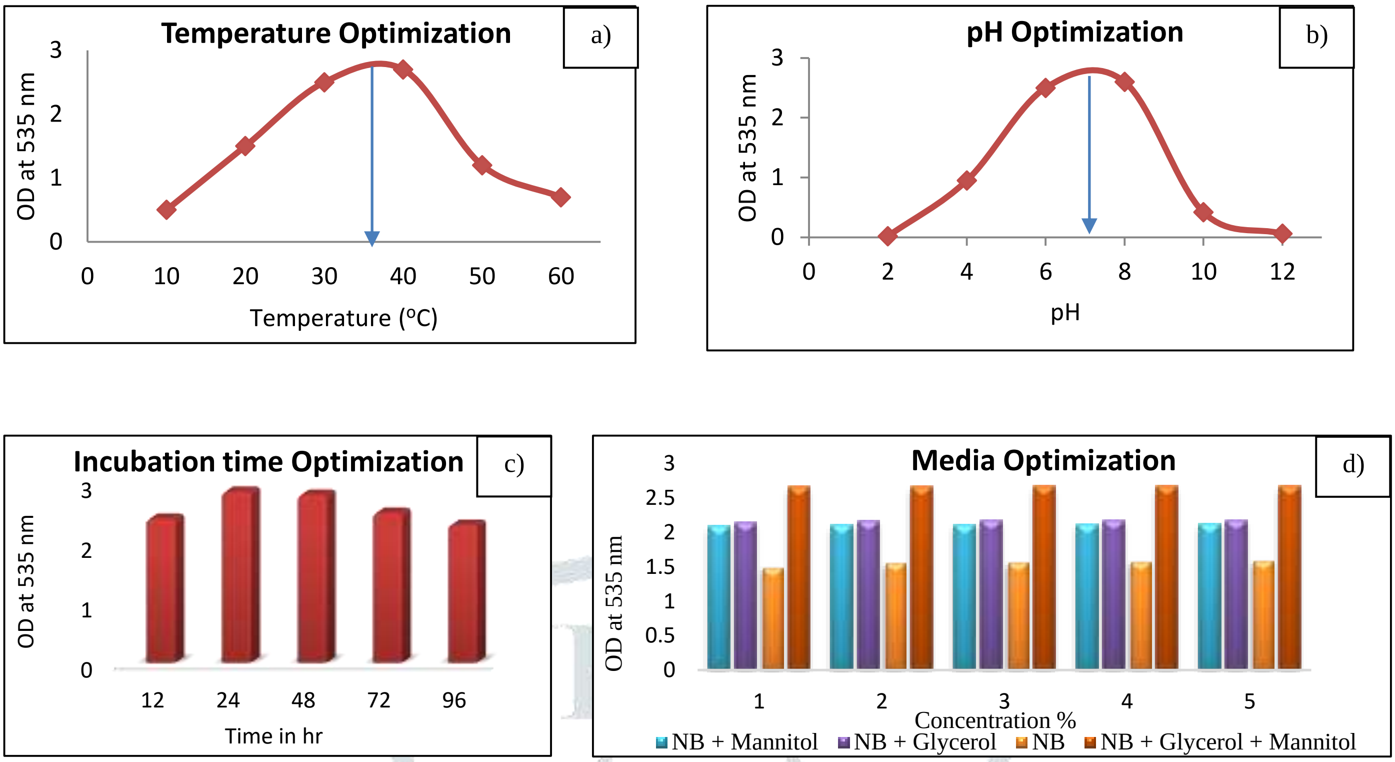

Figure 1: Optimization of production parameters. a) Temperature, b) pH, c) Time, d) Medium components

Figure 2: Confirmative test of prodigiosin

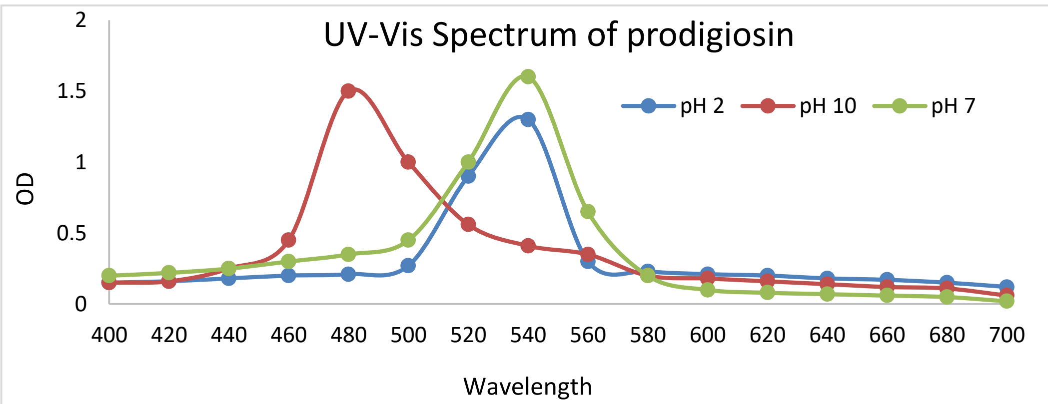

Figure 3: UV- Vis spectrum of the pigment at pH 2, 7 and 10.

Figure 4: Thin layer chromatogram of the extracted pigment

3.4 Dyeing different textile fabrics

White-colored cotton, terry-cotton, satin and woolen fabric were dyed with the extracted pigment, pigment + thiourea, and pigment + tartaric acid. Cotton, Terry-cotton and Woolen fabric was readily stained with the pigment, while satin fabric was poorly stained. After drying, the cotton fabric stained with pigment along with the mordant appeared bright followed by terry-cotton fabric and woolen fabric (Fig. 5).

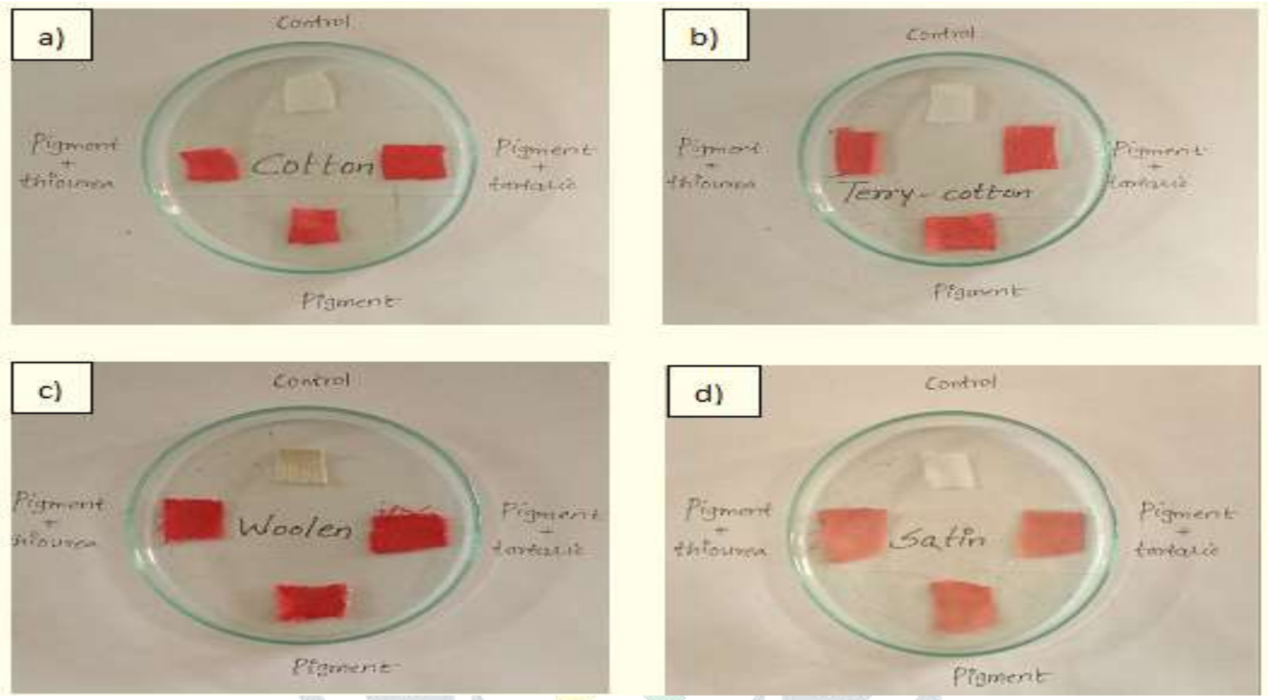

Figure 5: Fabric materials after dyeing with extracted pigment prodigiosin.
a)Cotton fabrics, b)Terry-cotton fabric, c)Woolen fabric, d)Satin fabric. The fabric pieces in the Petri dishes are dyed with- top fabric (control-no dye), bottom fabric (pigment), left fabric (Pigment + thiourea), right fabric (Pigment + tartaric acid)

3.5 Wash performance of the dyed fabrics

The absorbance of the detergent solution in which the dyed fabrics were incubated for 30 mins was measured at 535 nm. The cotton, terry-cotton and woolen fabric dyed without any mordant showed some loss of the pigment, while for dyed satin fabric, both with and without the mordant; there was a heavy loss of pigment (Fig. 6). Though cotton, terry-cotton and woolen fabric dyed using mordant lost some pigment on washing, the color of the fabric did not get fade, but without the mordant there was a considerable loss of the dye as well as the fabric became fade (Fig. 7).

In the case of Cotton and Terry-cotton, thiourea was found to be an ideal mordant and for woolen fabric, both thiourea and tartaric acid was efficient. Since satin fabric couldn't be dyed efficiently, as per the protocol; the method needs to be revised using any other mordant.

3.6 Antimicrobial activity of the dyed fabrics

The dyed fabric materials of cotton, terry-cotton, woolen and satin, were cut into pieces of 10mm x 10mm squares and they were aseptically placed onto the nutrient medium agar surface which was seeded with the test isolates, *Candida albicans, Staphylococcus aureus, and Pseudomonas aeruginosa.* The plates were incubated at 37 ºC for 24 hr. After the incubation period, the diameter of the zone of inhibition around the fabric was measured and recorded (Fig. 8).

Table 1 shows the antimicrobial activity of the dyed fabrics against the test isolates. Cotton and terry-cotton fabric dyed with prodigiosin was found to have good broad-spectrum antimicrobial activity against Gram-positive, Gram-negative and yeast, while woolen fabric had least activity. The satin fabric didn't show any antimicrobial activity.

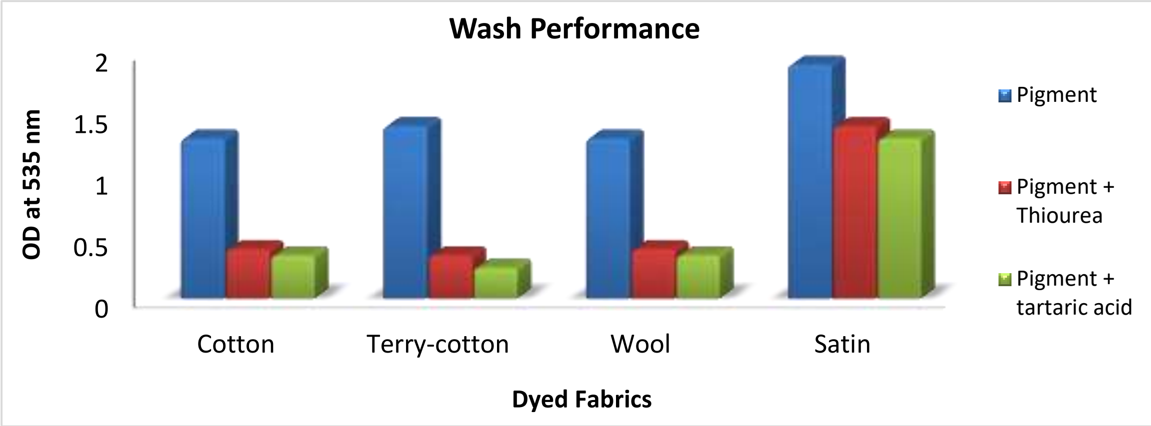

Figure 6: Absorbance of the detergent solution after washing the dyed fabric

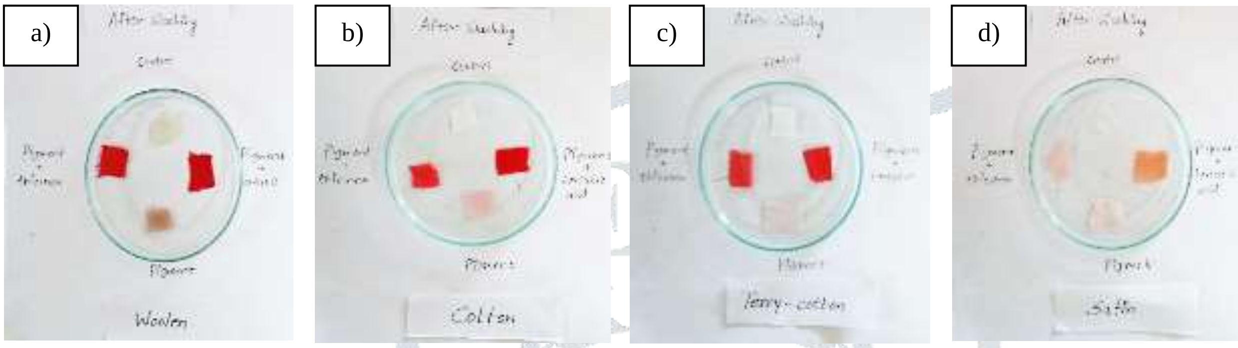

Figure 7: Fabric materials after washing with a detergent solution.
a)Woolen fabrics, b)Cotton fabric,c)Terry-cotton fabric, d)Satin fabric. The fabric pieces in the Petri dishes are those dyed with- top fabric (control-no dye), bottom fabric (pigment), left fabric (Pigment + thiourea), right fabric (Pigment + tartaric acid) before washing.

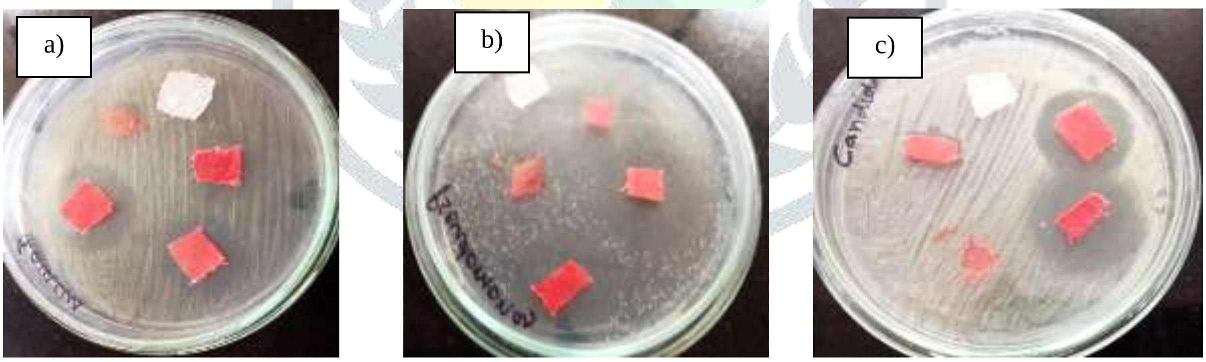

Figure 8: Antimicrobial activity of the fabric materials after dyeing with extracted pigment prodigiosin against a)Candida albicans, b) Staphylococcus aureus, c) Pseudomonas aeruginosa.

4. CONCLUSION

The red-pigment prodigiosin obtained from the bacteria *Serratia marcescens* showed significantly good application as a natural dye for coloring of cotton, terry-cotton, and woolen fabric. The fabric dyed with the pigment also showed broad-spectrum antibacterial property against skin infection-causing pathogens enhancing its business worth. The application of this natural and eco-friendly pigment will be economical as well as it will promote consumer's health protection from harmful effects of synthetic dye.

Table 1: Antimicrobial activity of the dyed fabrics against the test isolates.

Sr. no.	Microbial test culture	Dyed fabric material	Diameter of zone of inhibition (mm)
1.	*Staphylococcus aureus*	Cotton	25
		Terry- cotton	22
		Woolen	9
		Satin	-
2.	*Pseudomonas aeruginosa*	Cotton	30
		Terry- cotton	26
		Woolen	7
		Satin	-
3.	*Candida albicans*	Cotton	28
		Terry- cotton	23
		Woolen	5
		Satin	-

5. REFERENCES

1] Aberoumand, A. (2011). A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *WJDFS*, *6*(1), 71-78.

2] Amchova, P., Kotolova, H., &Ruda-Kucerova, J. (2015). Health safety issues of synthetic food colorants. *Regulatory Toxicology and Pharmacology*, *73*(3), 914-922.

3] Cohen, P. R. (2007). Community-acquired methicillin-resistant Staphylococcus aureus skin infections: a review of epidemiology, clinical features, management, and prevention. *International journal of dermatology*, *46*(1), 1-11.

4] Dąbrowska, M., Sienkiewicz, M., Kwiatkowski, P., & Dąbrowski, M. (2019). Diagnosis and treatment of mucosa Candida spp. infections – a review article. *Annales Universitatis Mariae Curie-Sklodowska, section C – Biologia*, *73* (1), 61.

5] Gerber, N. N., and Lechevalier, M. P. (1976). Prodiginine (prodigiosin-like) pigments from Streptomyces and other Actinomyces.

6] He, J., &Giusti, M. M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annual review of food science and technology*, *1*, 163-187.

7] Khan, S., & Malik, A. (2014). Environmental and health effects of textile industry wastewater. In *Environmental deterioration and human health* (pp. 55-71). Springer, Dordrecht.

8] Krishna, J. G. (2008). Pigment production by *marine Serratia sp. BTW J8* (Doctoral dissertation, Ph. D. Thesis. Department of Biotechnology, Cochin University of Science and Technology, Cochin, Kerala, India).

9] Patil, C. D., Patil, S. V., Salunke, B. K., &Salunkhe, R. B. (2011). Prodigiosin produced by Serratia marcescens NMCC46 as a mosquito larvicidal agent against Aedesaegypti and Anopheles stephensi. *Parasitology Research*, *109*(4), 1179-1187.

10] PB, S., JC, A., Rathinamala, J., &Jayashree, S. (2019). The role of red pigment prodigiosin from bacteria of earthworm gut as an anticancer agent. *Journal of Microbiology, Biotechnology, and Food Sciences*, *2019*, 246-251.

11] Rakh, R. R., Dalvi, S. M., Musle, B. B., &Raut, L. S. (2017). Production, Extraction, and Characterization of Red Pigment Produced by Serratia rubidaea JCM 1240T isolated from Soil. *Int. J. Curr. Microbiol. App. Sci*, *6*(1), 143-154.

12] Saxena, S., & Raja, A. S. M. (2014). Natural dyes: sources, chemistry, application, and sustainability issues. In *Roadmap to sustainable textiles and clothing* (pp. 37-80). Springer, Singapore.

13] Siebenhaar, F., Syska, W., Weller, K., Magerl, M., Zuberbier, T., Metz, M., & Maurer, M. (2007). Control of Pseudomonas aeruginosa skin infections in mice is mast cell-dependent. *The American journal of pathology*, *170*(6), 1910-1916.

14] Venil, C. K., Zakaria, Z. A., & Ahmad, W. A. (2013). Bacterial pigments and their applications. *Process Biochemistry*, *48*(7), 1065-1079.

15] Vijayarani, K., Gowralkar, M., Sesh, P. S. L., Savithri, M., & Kumanan, K. (2013). A preliminary study on the anti neoplastic effect of prodigiosin produced by Serratia marcescens isolated from termite (Odontotermes formosanus). *Indian Journal of Veterinary Pathology*, *37*(2), 195-198.