Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.22, 2016

Natural Dyeing of Silk and Cotton Fabric with Red Pigment from Penicillium purpurogenum which is Isolated from Goat Milk Contaminated Soil

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The research is financed by Directorate of research and Community service (DRPM) of Indonesia

Abstract
The use of much synthetic dye in dyeing industry resulted in dye containing wastewater which increases the environmental pollution. Therefore, the use of synthetic dye in industries must be replaced with eco-friendly non toxic dyes. The present study is an attempt to explore the posibility of water soluble red pigment of *Penicillium purpurogenum* as colorant for silk and cotton fabric. The red pigment was produced in potato-dextrose broth at a temperature of 30ºC in 14 days under a static condition. Red pigment extract was evaluated by dyeing on silk and cotton fabric. The effect of process parameters of dyeing such as dye absorption to silk and cotton and fastness to washing and ironing have been studied. In addition, red pigment extract also analyzed by using LC-MS method. The pigment show high affinity with silk and cotton fabric and is found to give good color fastness to washing and ironing (rating 4). LC-MS Results revealed that red pigment extract obtained protein compound complex. Natural red pigment extract has a significant potential for new dyeing techniques and will provide useful alternatives to synthetic dyes
Keywords: Red pigment, *Penicillium purpurogenum*, silk and cotton fabric

1. Introduction

In the recent years, environmental protection has become a challenge for the textile industry because it utilizes a lot of chemicals and synthetic dyes in dyeing process. Increased used of synthetic dyes and chemicals in the textile industry has resulted in the generation of a large quantities of effluent contain high level of dyes and toxic materials. The presence of colored effluent can inhibit the penetration of sunlight into the water body causing lack of oxygen dissolved that triggers the anaerobic microbial activity that produces a foul smell. Environmental protection is done by reducing the use of synthetic colorants and replacing it with natural colorants or pigment. Most natural pigments are extracted from plant like annatto, grapes, paprika, etc. and microorganisms like bacteria, fungi and algae. Numerous studies have reported the application of plant-based pigment in dyeing processes includes tamarind fruits fods (Umar, I.A. 2013), clitoria ternotea flower (Lakshmi, L. 2015), marigold flower (Rajeswari Devi V. *et al.* 2015) and *Plumeria rubra* L flower (Narayana Swamy, *et al* . 2016). The accessible authorized natural pigments from plant have numerous limitations such as instability against light, heat, adverse pH and are often non-availability throughout the year. Hence, it is suggested to exploit the potential of other biological source of pigment such as fungi, bacteria and algae. Natural pigments such as anthraquinone, anthraquinone carboxylic acids, pre-anthraquinones are extracted from filamentous fungi. The application of fungal pigments in dyeing of cotton, silk and wool has been reported in several studies. Pigments of fungal strains *Acrostalagmus* sp., *Alternaria* sp., *Bisporomyces* sp., *Phymatotricum* sp., *Penicillium chrysogenum*, *Penicillium italicum*, *Penicillium oxalicum*, and *Penicillium regulosum* produce a reddish brown color pigments reported fastness to washing and exposure to UV light. (Mabrouk, A.M. 2011). In addition, *Curvalaria lunata*, *Trichoderma virens* and *Alternaria alternata* produce a red pigment used for dyeing silk and wool (Sharma *et al,* 2012). Fungus *Thermomyces sp*. also reported to produce pigment yellow color does not fade to sunlight, washing and scraping after attempted for dyeing cotton, silk and wool (Poorniammal*, et al.* 2013). The advantages natural pigment production from fungal include easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shade. The present study aimed to assess the potentiality of red pigments produced by *Penicillium purpurogenum* to dye silk and cotton fabrics. A *Penicillium purpurogenum* strain was isolated from goat milk contaminated soil of Buleleng, Bali, Indonesia. The application was investigated by studying the physiochemical parameters of the pigment such as absorption percentage of pigment to fabrics and color fastness to washing and heating. Besides that, the main compound of red pigment also identified by LC-MS.

2. Material and Methods

2.1 Materials

Chemicals and textile material: Laboratory grade chemicals, dextrose, glucose, yeast extract. Textile material such as silk and cotton fabric were used for dyeing process.

Microorganism **:** The microorganism used in the study was a red pigment producing fungus identified as *Penicillium purpurogenum* isolated from goat milk contaminated soil of Sepang village, Buleleng-Bali, Indonesia. The fungus was identified by looking at the typical appearance of spores under microscope. Fungus of *Penicillium purpurogenum* culturised on potatoes dextrose agar medium (PDA) at pH 5 and 4°C and subculturised every 4 weeks.

2.2. Methods

2.2.1 Production and Extraction of Red Pigment

The *Penicillium purpurogenum* was grown in sterile PD broth containing 2% glucose, 2% yeast extract, grated coconut pulp as the supporting medium for 14 days with shaking conditions at 120 rpm. The growth medium was maintained at 30°C and pH 5. Water soluble red pigment was filtered through Whattman filter paper No.1 and the filtrate was centrifuged at 1000 rpm for 15 minutes to separate the pigment from biomass.

2.2.2 Pigment Purification and Analysis of Main compound by LC-MS Method

Purification of red pigment on column chromatography using silica gel 60 G as a stationary phase and a mixture of methanol: glacial acetic acid: water at a ratio 18 : 1: 1 as a mobile phase. Amount 10 mL of red pigment transferred into the column and then eluted using a mobile phase. The yellow fraction was eluted first, followed by red fraction. Only red pigment fraction was analyzed of main compound by liquid chromatography-mass spectrophotometer (LC-MS).

2.2.3 Stability Test for Red Pigment

Stability test of red pigment was carried out according to the method reported by Perumal *et al.* (2009). Amount 10 mL of red pigment extract was taken in a test tube and incubated in a water bath set at 40, 50, 60, 70, 80, and 90°C for 15 min. After incubation, it was cooled and subjected to analysis using an ultraviolet-visible spectrophotometer at 490 nm. Another set of test tubes each containing 10 ml of the red pigment extract was adjusted to pH values of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, then incubated for 15 min and measured pigment absorbance using an ultraviolet-visible spectrophotometer at 490 nm.

2.2.4 Application of Red Pigment on Dyeing

Lab scale dyeing experiment were carried out on silk and cotton fabrics. The samples were cutted into small pieces (approximately 10 x 10 cm). Dyeing process consisted of three steps includes mordanting, dyeing and fixation. Mordanting was carried out by immerses silk and cotton separately in a beaker glass containing 50 ml of alum as mordant (5%) then boiled for 60 minutes. The silk and cotton samples were dyed directly with 100 mL of red pigment extract for 2 hours at 70-80^{o}C. After dyeing, the temperature was lowered to room temperature, then the fabrics samples rinsed and fixed in 2% of lemon fruit solution for 30 min then rinsed and dried.

2.2.5 The Affinity of Red Pigment on Silk and Cotton Fabric

Test absorption of silk and cotton fabric of the red pigment is done by measuring the absorbance of the pigment solution before and after immersion at a wavelength of 490 nm using a UV-Vis spectrophotometer. The percentage absorption of the dyed fabric was calculated using the formula:

$$Percentage \quad of \quad dyed \quad sample \quad = \frac{A_o - A_1}{A_o} x\, 100\ \%$$

Where A_o the absorbance of red pigment before treatment and A_1 the absorbance of red pigment after treatment at λ_{max} of the pigment used.

2.2.6 Color Fastness Testing

2.2.6.1 Color Fastness to Washing

The samples of dyed fabric were immersed into 50 ml of neutral solution of a synthetic detergent (5 g/L sodium lauryl sulphate). The test was run for 30 min while shaking, samples were then removed, rinsed then dried. The change in color of the dyed fabrics are evaluated with gray scale standard.

2.2.6.2 Color Fastness to Heating

Testing of color fastness to heat treatment was done by placing the colored fabric sample on the oven at a temperature of 130^{o}C for 5 minutes for silk for while at 210^{o}C for cotton fabrics. Color fastness of silk and cotton were evaluated with gray scale standard

3. Results and Discussion

3.1 Pigment Production

The fungi *Penicillium purpurogenum* chosen in present study produced red pigment in submerged culture. The culture medium, temperature and incubation time for *Penicillium purpurogenum* was potato dextrose broth containing 2% glucose, 2% yeast extract and grated coconut pulp as the supporting medium 5 at 30^{o}C for 14 days with shaking conditions at 120 rpm. The pH culture medium for red pigment production was 5. The red pigment was extracted with 100 mL of distilled water and then filtered with Whattman filter paper. The filtrate was centrifuged at 1000 rpm for 15 minutes and the supernatant was added distilled water to a final volume of

500 mL. Red pigment in culture medium was shown in Figure 1.

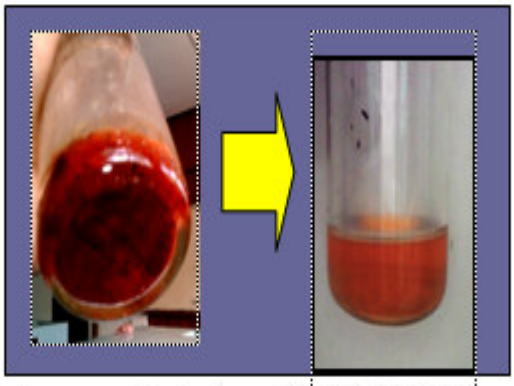

Figure 1. Performance of red pigment from *Penicillium purpurogenum*

3.2 Purification and Determination Main Colored Component Using LC-MS Method

The crude pigment extract was purified on column with a silica gel 60 G as stationary phase while mixture of methanol-glacial acetic acid-water ratio of 18: 1: 1 as mobile phase. Separation of pigment on column chromatography is fractionated in to two different fractions (figure 2).

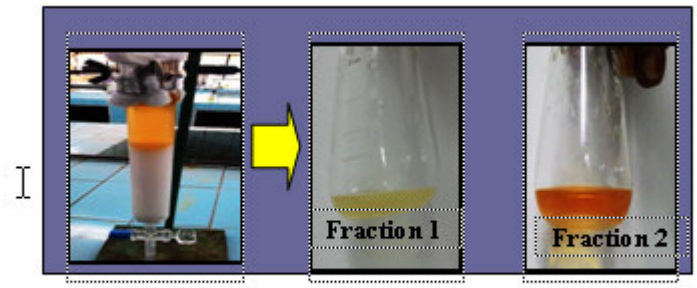

Figure 2. Separation of red pigment fraction on a column of silica gel 60 G

The crude pigment extract of *Penicillium purpurogenum* is fractionated in to two different major fraction by column chromatography. The first yellow fraction (fraction 1) was eluted with eluent consist of methanol-glacial acetic acid-water (18: 1: 1) followed by the second red fraction (fraction 2) with the same eluent. The main colored component of red pigment fraction was identified by LC-MS method. The LC-MS spectra of red pigment was presented in Figure 3.

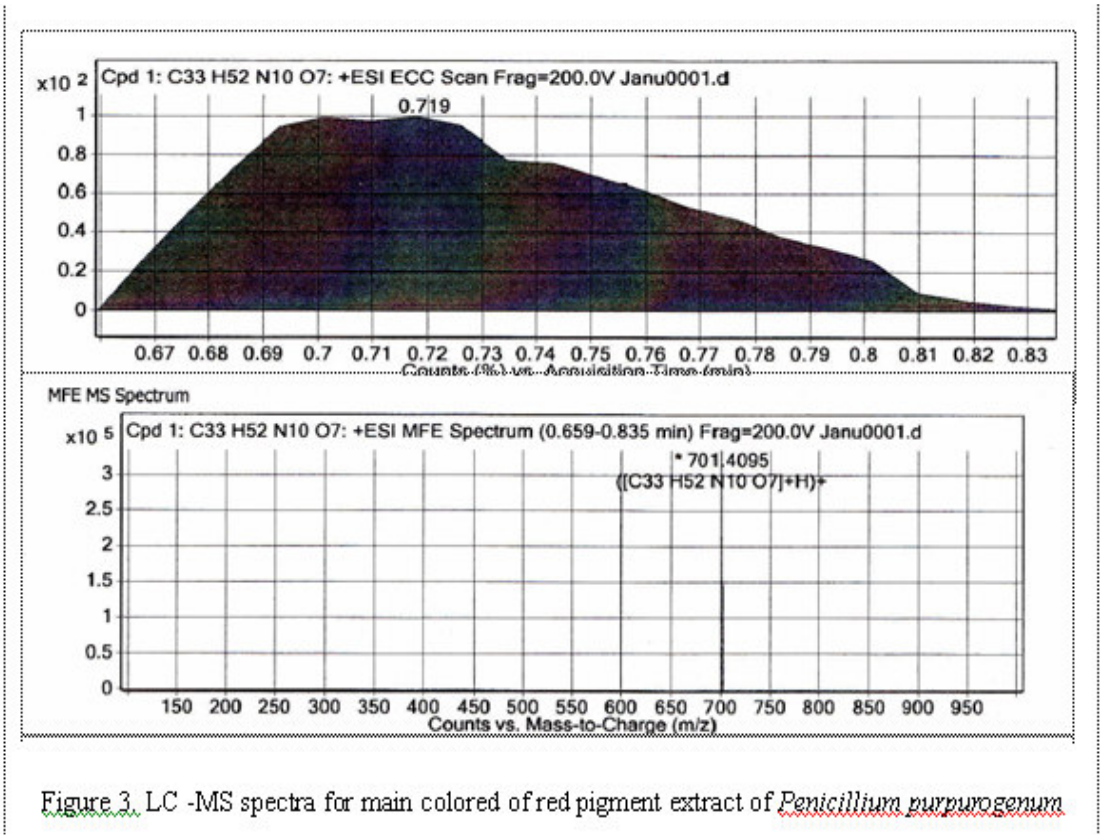

Figure 3. LC -MS spectra for main colored of red pigment extract of *Penicillium purpurogenum*

Figure 3 shows the LC-MS spectra showed that the main colored component of red pigment from *Penicillium purpurogenum* has the formula $C_{33}H_{52}N_{10}O_7$ with a retention time of 0.719 and the value of m / z was 701.4095. This compound is believed to be the protein complex.

3.3 Stability of Pigment

Stability of Pigment extract from *Penicillium purpurogenum* in different pH and temperature was presented in figure 4-5.

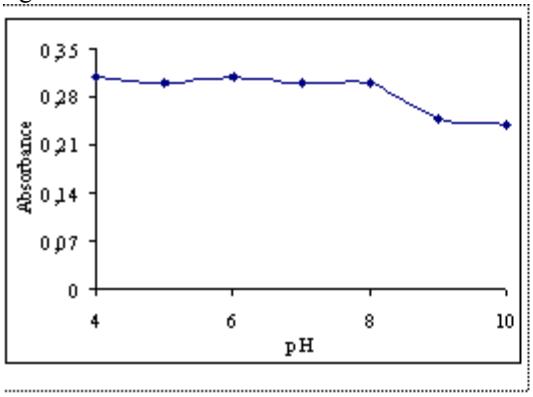

Figure 4. Stability of red pigment on pH treatment

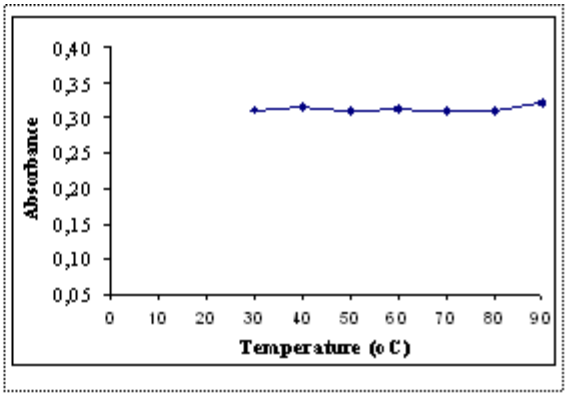

Figure 5. Stability of red pigment on temperature

The effect of pH and temperature on color stability of fungal pigment were studied by several authors. In this research, all the experiments were performed in a different temperature (30-90ºC) and different pH (4-10), then after 15 minutes incubation, the absorbance was recorded at 490 nm. Absorbance of red pigment was stable at 30-80°C and pH 4-8. The stability of pigment was supported by the research results conducted by Wang Hailei, *et al* (2011) who reported that the color of red pigment from *Penicillium sp*. HSD07B remained relatively stable at the heating temperature of 100ºC and treatment of pH 2 to pH 10. Devi, S. *et al.* 2012 reported that betacyanin extracts of *Basella alba* fruit were highly or moderately resistant to the pH and temperature. Wang, J. *et al*.2013 also reported that the stability of anthocyanin from black-skinned peanuts decreasing with increasing pH(2-10), anthocyanin stable at pH 5 to 7. Based on the research result, fungal pigment more stable than plant-based pigment under variation of pH and temperature treatment.

3.4 Application of Pigment on Dyeing

Red pigment extract was applied for coloring silk and cotton fabric. Sightings fabrics before and after dyed were presented in Figure 6.

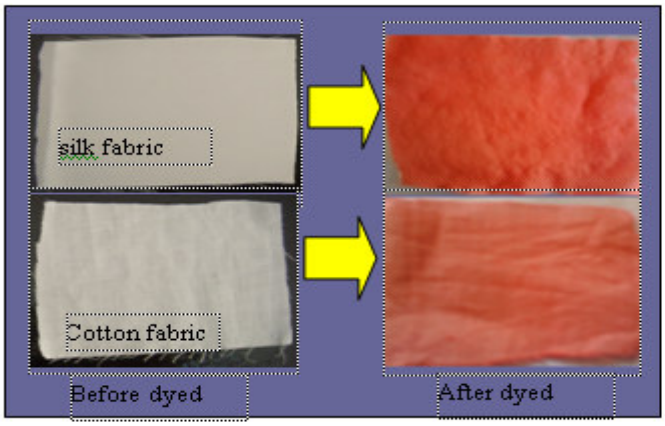

Figure 6. The appearance of silk and cotton before and after dyed with red pigment

Quantitative analysis of the absorption of silk and cotton to the red pigment is done by measuring the difference in absorbance of the solution before and after dyed. The results of absorbance measurements using UV-Vis spectrophotometer at wavelength of 490 nm are presented in Table 1.

Table 1. Percentage absorption of red pigment extract on silk and cotton fabrics

Fabric	Absorption		(%)
	Before dyed	After dyed	
Silk	0.611	0.217	64.48
Cotton	0.611	0.243	60.23

Table 1 gives the absorbance of red pigment extract and their corresponding percentage absorption for silk and cotton. As can be seen, percentage absorption of red pigment extract is 64,48% for silk and 60,23% for cotton respectively. As expected silk has percentage absorption higher than cotton. The percentage absorption of some pigments also carried out by Sharma *et al*. (2012). In their study was reported that the percentage absorption of *Trichoderma virens* pigment in silk and wool up to 44.58% and 48.37%, whereas *Curvularia lunata* fungal pigment up to 53.34% and 54.47%, and Alternaria alternata fungal pigmet up to 53.60% and 55.02%. Fastness properties is intended to assess the strength of the bond between the fabric with a pigment. The color fatness of silk and cotton revealed that dyed samples have good fastness to washing and heating. The result of color fastness properties of red pigment to washing and heating is in line with the results of research by Sharma *et al*. (2012), reported that coloring silk and wool using pigments of *Trichoderma virens*, *Curvularia lunata* and *Alternaria alternata* have excellent fastness to washing.

4. Conclusions

From the results it can be concluded that the red pigment extract of *Penicillium purpurogenum* were moderately stable in temperature at 40-80^{o}C and at pH 4-9. Color fastness properties of silk and cotton to washing and heating treatment was good category, while the percentage absorption of red pigment on silk higher than cotton fabric. The red pigment extract of *Penicillium purpurogenum* composed by main compound with molecular formula $C_{33}H_{52}N_{10}O_7$. This molecule believed to be the protein complex with retention time of 0.719 and value of m / z of 701.4095. Thus these results suggest that red pigment extract of *Penicillium purpurogenum* could be source for textile colorant.

References

Devi, S., Reshmi, S.K., Aravindhan, K.M., & Devi, P.S. (2012). The Effect of Light, Temperature, pH on Stability of Betacyanin Pigments in Basella Alba Fruit. *Asian Journal of Pharmaceutical and Clinical Research,* 5, 107-110.

Lakshmi, L. (2015). Studies on Natural Dyeing Obtained from Clitoria Ternotea Flowers for Dyeing Cotton and Silk in Different Conditions. *International Journal of Innovative Research in Science Engineering and Technology,* 4, 80-84.

Mabrouk, A.M., El-Khrisy, E.A.M., Youssef, Y.A., & Asem, M.A. (2011). Production of Textile Reddish

Brown Dyes by Fungi, *Malaysian Journal of Microbiology,* 7, 33-40.
Narayana Swamy, Ninge Gowda, K.N., & Sudhakar, R. (2016). Extraction and Dyeing Conditions of Natural Dye from Flowers of *Plumeria rubra* L. on Textiles and Fastness Properties. *Indian Journal of Traditional Knowledge*, 15, 278-284.
Perumal, K., Stalin, V., Chandrasekarenthiran, S., Sumathi, E., & Saravanakumar, A. (2009). Extraction and Characterization of Pigment from Sclerotinia sp. and Its Use in Dyeing Cotton. *Journal of Textile Research,* 79, 1178 – 1187.
Poorniammal, R., Parthiban, M., Gunasekaran, S., Murugesan, R. & Thilagavathi, G. (2013). Natural dye production from *Thermomyces* sp. fungi for textile application. *Indian Journal of Fibre & Textile Research,* 38, 276-279.
Rajeswari Devi, V., Chandan Kumar Jha., Ratan Kumar., & Venkat Kumar, S. (2015). Extraction of Natural Dyes from Marigold Flower (*Tageteserecta*) and Dyeing of Fabrics and Yarns: A Focus on Colorimetric Analysis and Fastness Properties. *Der Pharmacia Lettre,* 1, 185-195.
Sharma, D., Gupta C., Aggarwal, S., & Nagpal, N. (2012). Pigment Extraction from Fungus for Textile Dyeing. *Indian Journal of Fibre & Textile Research,* 3, 68-73.
Umar, I.A. (2013). Fastness Properties of Colorant Extracted from Tamarind Fruits Fods to Dye Cotton and Silk Fabrics. *Journal of Natural Sciences Research,* 3 .60-66
Wang Hailei,, Ren Zhifang,, Li Ping,, Gu Yanchang,, Liu Guosheng,, & Yao Jianming. (2011). Improvement of The Production of a Red Pigment in *Penicillium* sp. HSDO7B Synthesized During Co-culture with *Candida tropicalis*. *Bioresource Technology*, 102, 6082-6087.
Wang, J., Shen, X., & Chen, Y. (2013). Effect of pH, Tempearture and Iron on The Stability of Anthocyanins from Black-Skinned Peanuts (*Arachis hypogaea* L). *African Journal of Agricultural Research,* 8, 2044-2047.