NC STATE UNIVERSITY

JTATM

JOURNAL OF TEXTILE AND APPAREL, TECHNOLOGY AND MANAGEMENT

Volume 5, Issue 4, Fall 2007

ANTIBACTERIAL ACTIVITY AND UV PROPERTY OF SHIKONIN ON SILK SUBSTRATE

Renuka Dhandapani and Ajoy K. Sarkar
Department of Design and Merchandising
Colorado State University, Fort Collins, CO 80523
E-mail: sarkar@cahs.colostate.edu

ABSTRACT

Shikonin, a naphthoquinone found in the herb Lithospermum erythrorhizon has been used as a red dye for centuries and is reported to possess medicinal properties. In the current study, shikonin was evaluated as a multi-functional antibacterial and UV protective agent on a silk fabric. Antibacterial activity against Staphylococcus aureus and Escherichia coli was analyzed qualitatively in terms of zone of inhibition and quantitatively in terms of percentage reduction in bacteria. Effectiveness of shikonin against ultraviolet radiation was evaluated in terms of Ultraviolet Protection Factor values. Durability of shikonin to laundering and exposure to light were also studied. Results showed significant antibacterial activity against the two bacteria. In addition, the UV protective property of treated silk substrate was considerably enhanced. Shikonin is also shown to retain its protective properties on laundering and light exposure. Natural agents such as shikonin are viable additions to the arsenal of multi-functional agents for textile substrates.

Keywords: Shikonin; Naphtoquinone; Antibacterial activity; Staphylococcus aureus; Escherichia coli; UPF

1. Introduction

According to the Centers for Disease Control and Prevention, Department of Health and Human Services, USA about two million people contact infections in hospitals each year. The infections are spread via person-to-person contact and also via surface contact with hands, clothes, and hospital devices such as surgeon gowns and bedclothes [1]. Among the disease causing bacteria in hospitals, *Escherichia coli* and *Staphylococcus aureus* were identified to be the two most common bacterial isolates responsible for cross infecting large number of patients [2]. Compounding the problem is the fact that the diseases caused by these bacteria are increasingly not treatable as the bacteria have developed resistance to currently available therapies.

A degree of protection from the infection causing bacteria can be obtained by using appropriate protective clothing such as face masks and gloves but clothing by itself has been found to be inadequate in preventing transmission of disease [3]. In addition, bacteria can survive on textiles for many days and contribute in transmission of disease [4]. A better level of protection is obtained by using antibacterial finished textile products. Examples of agents that have been successfully used as antibacterial agents on textile substrates include silver [5,6], 2,4,4'- Trichloro-2' –hydroxydiphenyl ether [7,8] and quaternary ammonium chloride [9,10]. However, over a period of time bacteria generally tend to develop resistance to almost all agents rendering them ineffective for extended use. Thus, it is prudent that we seek innovative sources of antibacterial agents in order to battle the growing problem of antibacterial resistant bacteria. Natural dyes derived from plant sources have long been known to possess medicinal properties and consequently it seemed logical to explore the antibacterial activity of dyes. Among the several dyes that are currently being investigated in our laboratory, shikonin was particularly promising during initial screening which was not surprising since it is reported to possess significant antibacterial, anti-inflammatory and antitumor activities [11, 12, 13]. However, the efficacy of shikonin as an antibacterial agent on a textile substrate has not been investigated and is therefore the focus of this study.

2. Experimental

2.1. Materials

2.1.1. Substrate

Silk was chosen as the substrate as shikonin has traditionally been used to dye silk and has good affinity for the fiber. Characteristics of the silk substrate is given in Table 1. The fabric was obtained from TestFabrics Inc., PA, USA.

Table 1. Silk fabric characteristics

Fabric	**Weave**	**Thickness, mm**	**Weight, g/m^2**	**Warps/inch**	**Fillings/inch**
Silk	Plain	0.254	94	80	76

2.1.2. Shikonin

Shikonin (MW 288.3; Purity 97%) was obtained from EMD Biosciences, San Diego, CA, USA.

2.1.3. Test organisms

Staphylococcus aureus (ATCC® 6538, PML Microbiologicals, Wilsonville, OR, USA) and *Escherichia coli* (ATCC® 25922, PML Microbiologicals, Wilsonville, OR, USA) were the two microorganisms used in this study. *S. aureus* is a gram-positive bacterium and *E.coli* is a gram-negative bacterium. Standard microbiological procedures were employed to maintain cultures of the bacteria in the laboratory.

2.2. Methods

2.2.1. Dyeing

Fabric samples (15 cm x 15 cm) were dyed with shikonin using a post-mordanting method with alum as a mordant. Dye concentrations used were 0.10%, 0.25% and 0.5% on weight of fabric. Samples were introduced in the dye bath at room temperature at a material to liquor ratio of 1:40. Temperature of the dye bath was raised to boil and dyeing was continued at boil for 60 minutes followed by addition of 10% alum on weight of fabric. Dyeing was continued for another 45 minutes. Samples were then rinsed in deionized water and air dried. Three replications were done at each concentration of the dye. Undyed sample was used as negative control while a sample treated with 5% concentration (owf) of a commercial antibacterial agent served as positive control.

2.2.2. Qualitative evaluation

Five streaks of the microorganisms were inoculated on nutrient agar plates (peptone 5 g/L; beef extract 3 g/L; nutrient agar 15 g/L, NaCl 8 g/L; pH 6.8 ± 0.1). Fabric samples of size 25 mm x 50 mm were then placed in intimate contact with the bacteria inoculated agar. The plates were incubated for 24 h at 37 ºC. At the end of this period the plates were examined for presence of clear area of interrupted growth underneath and adjacent to the test fabric which gave an indication of the antibacterial activity of the fabric. Qualitative evaluation of dyed fabrics was done by calculating the zone of inhibition of the samples using equation 1. An inhibition zone greater than 2 mm was taken as an indication of good antibacterial effect.

$$W = \frac{(T - D)}{2} \qquad \text{Equation 1}$$

where:

W= width of clear zone of inhibition, mm

T= total diameter of test specimen and clear zone, mm

D= diameter of the test specimen, mm

2.2.3. Quantitative evaluation

Quantitative evaluation was done by enumerating the Colony Forming Units (CFU) using a Reichert Darkfield Quebec Colony Counter (Reichert Analytical Instruments, Depew, NY, USA) and calculating the percent reduction in bacteria using equation 2.

$$R = \frac{(B - A)}{B}.100 \qquad \text{Equation 2}$$

where:

R= percent reduction in bacteria
A= CFU for dyed fabric
B= CFU for undyed fabric

2.2.4. Durability of dyed fabrics and antibacterial activity to laundering

Dyed fabrics were subjected to laundering at 40 °C for 45 minutes in an Atlas Launder-O-Meter with a M:L ratio of 1:40. The antibacterial properties of the laundered fabrics were then quantitatively evaluated.

2.2.5. Durability of dyed fabrics and antibacterial activity to light

Dyed fabrics were subjected to light exposure by placing the samples in an Atlas Sun Test XLS+ weatherometer with the following parameters: Black Standard Temperature (BST) of 63 °C, phase time of 300 minutes, irradiance (E) of 500 W/m^2, and final dosage of 9000 kJ/m^2. The samples were exposed front and back in the chamber on successive days. The antibacterial properties of the light exposed samples were then quantitatively evaluated.

2.2.6. Ultraviolet Protection Factor (UPF)

UPF is the scientific term used to indicate the amount of Ultraviolet (UV) protection provided to skin by fabric. UPF is defined as the ratio of the average effective UV irradiance calculated for unprotected skin to the average UV irradiance calculated for skin protected by the test fabric. The higher the value, the longer a person can stay in the sun until the area of skin under the fabric becomes red. An effective UVR dose (ED) for unprotected skin is calculated by convolving the incident solar spectral power distribution with the relative spectral effectiveness function and summing over the wavelength range 290-400 nm. The calculation is repeated with the spectral transmission of the fabric as an additional weighting to get the effective dose (ED$_m$) for the skin when it is protected. The UPF is defined as the ratio of ED to ED$_m$ and calculated as follows:

$$UPF = \frac{ED}{ED_m} = \frac{\sum_{290nm}^{400nm} E_\lambda S_\lambda \Delta \lambda}{\sum_{290nm}^{400nm} E_\lambda S_\lambda T_\lambda \Delta \lambda} \qquad \text{Equation 3}$$

where:

E_λ = erythemal spectral effectiveness
S_λ = solar spectral irradiance in Wm^{-2}nm^{-1}
T_λ = spectral transmittance of fabric
Δ_λ = the bandwidth in nm
λ = the wavelength in nm

The UPF was measured using a labsphere® UV-100 F Ultraviolet Transmission Analyzer. Fabrics with UPF values greater than 40 are considered as having Excellent UV Protection, UPF values between 25 and 39 translate to Very Good UV protection and fabrics with values between 15 and 24 are considered as having Good UV protection.

3. Results and discussion

3.1. Qualitative determination of antibacterial activity of dyed fabrics

The mean zones of inhibition of silk substrate dyed with shikonin at various concentrations against *S.aureus* and *E.coli* is shown in Table 2.

Table 2. Mean zone of inhibition of silk dyed with shikonin against *S.aureus* and *E.coli*

Concentration (%)	Mean zone of inhibition (mm)	
	S.aureus	***E.coli***
0.10	11.5	3.3
0.25	12.2	3.5
0.50	13.2	3.5

The data in Table 2 clearly indicates dyed silk as having mean zones of inhibition far greater than 2 mm against the two bacteria. In addition, there was a complete lack of growth underneath the test fabrics. The dyed fabrics therefore possess excellent antibacterial activity. The lowest concentration of 0.10% (owf) can be considered sufficiently effective for silk substrate against the two bacteria as the zones of inhibition obtained at this colorant concentration is higher than the required minimum of 2 mm.

3.2. Quantitative evaluation of antibacterial activity of dyed fabrics

Quantitative evaluation was done to determine the percentage reduction in bacterial populations for shikonin dyed silk substrate as compared to untreated control and a positive control.

3.2.1. Quantitative evaluation of shikonin dyed silk against S.aureus

Antibacterial activity of shikonin treated fabrics and control fabrics after dyeing, after laundering and on exposure to light against *S.aureus* is shown in Table 3.

Table 3. Antibacterial activity of shikonin dyed silk against *S.aureus*

Fabric Samples		**Dyed**		**Laundered**		**Light exposed**	
		Number of CFU	Reduction in bacteria (%)	Number of CFU	Reduction in bacteria (%)	Number of CFU	Reduction in bacteria (%)
Untreated Control		760	____	204	___	222	___
Positive Control, 5% owf		4	99.5	23	88.7	48	78.4
Shikonin	0.10% owf	84	89.0	108	47.1	73	67.3
	0.25% owf	108	86.7	105	48.8	71.5	67.8
	0.50% owf	97	92.2	92	54.7	62	72.1

It is unmistakably evident from the data in Table 3 that shikonin dyed silk substrate has excellent antibacterial effectiveness against *S.aureus*. The results also show the antibacterial activity decreasing to a greater extent than the commercial antibacterial agent after laundering and on exposure to light. For example, the 0.10% owf dyed silk shows a reduction in antibacterial activity by 47% on laundering. Similarly, the antibacterial effectiveness reduces by 24% after exposure to light. However, the data also show that higher the concentration of shikonin in the dyed fabric a higher percentage of antibacterial activity is retained after laundering and light exposure. Therefore, the antibacterial activity of shikonin is bactericidal and not bacteriostatic.

3.2.2. Quantitative evaluation of shikonin dyed silk against E.coli

Antibacterial activity of shikonin treated fabrics and control fabrics after dyeing, after laundering and on exposure to light against *E.coli* is shown in Table 4.

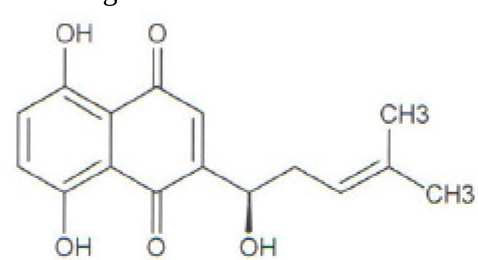
Table 4. Antibacterial activity of shikonin dyed silk against *E.coli*

Fabric Samples	**Dyed**		**Laundered**		**Light exposed**	
	Number of CFU	Reduction in bacteria (%)	Number of CFU	Reduction in bacteria (%)	Number of CFU	Reduction in bacteria (%)
Untreated Control	464	___	281	___	430	___
Positive Control, 5% owf	130	72.0	150	46.6	145	66.3
Shikonin 0.10% owf	19	95.9	43	84.7	222	48.4
Shikonin 0.25% owf	28	94.0	39	86.1	173.5	59.7
Shikonin 0.50% owf	42	91.0	30	89.2	162	62.3

Table 4 shows some interesting facts regarding the effectiveness of shikonin against *E.coli*. First, shikonin displays higher antibacterial activity against *E.coli* than the commercial antibacterial agent at all concentrations of dye. Second, a higher degree of the antibacterial activity of shikonin (88%) is retained after laundering. The decreasing trend in the antibacterial activity of shikonin after light exposure is similar to the results obtained against *S.aureus*. The reason could be that shikonin is susceptible to photooxidation on exposure to light [14].

The excellent antibacterial activity of shikonin as demonstrated by both qualitative and quantitative studies can be explained on the basis of its structure depicted in Figure 1.

Figure 1. Structure of shikonin



Shikonin is the major constituent of the red pigment extracts from the roots of the plant *Lithospermum erythrorhizon*.

Structure-activity studies by Papageorgiou and co-workers [14] have shown that the antibacterial effect is due to the naphthoquinone pigment. Naphthoquinones are thought to function as an antibacterial by targeting surface-exposed adhesions, cell wall polypeptides, and membrane bound enzymes in the microbial cell leading to inactivation of protein and thus a loss of function [15].

3.3. UV protective properties of silk dyed with shikonin

The Ultraviolet Protection Factor values measured for shikonin dyed, laundered, and light exposed fabrics are listed in Table 5.

Table 5. UPF values of shikonin dyed, laundered, and light exposed silk substrate

Fabrics		Dyed	Laundered	Light exposed
Silk	Untreated	15.4	22.4	26.1
	0.10% owf	36.8	41.6	> 50
	0.25% owf	44.2	> 50	> 50
	0.50% owf	> 50	> 50	> 50

The UPF values obtained for the untreated and treated substrate indicate that silk fabric even without any treatment provides Good UV protection (UPF= 15.4). However, treatment with shikonin dramatically enhances the UV protective property of the substrate. The UPF values increase from Good (untreated) to Very Good (0.10% owf) to Excellent (0.25% owf and 0.50% owf) category. The UV property of shikonin dyed fabric is, therefore, a function of concentration of dye. More interestingly, the UPF values further increased on laundering and on exposure to light. This increase in UPF value is attributed to the change to darker hue of the dyed fabrics. Darker colored fabrics have higher UPF values and attendant higher protection against UV radiation than lighter shades.

4. Conclusions

Shikonin was found to be an effective antibacterial agent against a gram-positive bacterium as well as a gram-negative bacterium at low concentrations of the dye. In addition, shikonin imparted excellent UV protection to the substrate. Shikonin therefore has the potential to act as a multi-functional agent with both antibacterial and anti-ultraviolet properties. The results of this study are promising since they open the prospect of a new line of agents and defense against common infection causing bacteria. However, it should be noted that to effectively combat nosocomial infections a bacterial reduction of greater than 95% after 100 commercial launderings is required. Therefore, additional studies are needed to improve the fixation and durability of shikonin on textile substrates.

Acknowledgements

This research was supported by the U.S. Department of Agriculture Multi-State Research Project S-1026 (Textile Materials and Technologies addressing Energy, Health and other National Security Issues) via a grant by the Colorado Agricultural Experiment Station.

References

[1] Ayliffe G.A., & Lowbury E.J. (1982). Airborne infection in hospital. *Journal of Hospital Infection, 3*(3), 217-240.

[2] Bozja, J., Sherrill, J., Michielsen, S., & Stojilljkovic, I. (2003). Porphyrin-based light-activated antimicrobial materials. *Journal of Polymer Science, 41*, 2297-2303.

[3] Sun, G., & Worley, S.D. (2005). Chemistry of durable and regenerable biocidal textiles. *Journal of Chemical Education, 82*, 60-64.

[4] Neely, A.N., & Maley, M.P. (2000). Survival of Enterococci and Staphylococci on hospital fabrics and plastic. *Journal of Clinical Microbiology, 38*(2), 724-726.

[5] Haug, S., & Roll, A., Schmid-Grendelmeier, P., Johansen, P., Wuthrich, B., Kundig, T.M., & Senti, G. (2006). Coated textiles in the treatment of atopic dermatitis. *Current Problems in Dermatology, 33*, 144-151.

[6] Lansdown, A. (2004). A review of the use of silver in wound care: facts and fallacies. *British journal of nursing 2004, 13*(6), S6-S19.

[7] Kramer, A., Guggenbichler, P., Heldt, P., Junger, M., Ladwig, A., Thierbach, H., Weber, U., & Daeschlein, G. (2006). Hygienic relevance and risk assessment of antimicrobial-impregnated textiles. *Current Problems in Dermatology, 33*, 78-109.

[8] McMurry, L.M., McDemott, P.F., & Levy, S.B. (1999). Genetic evidence that InhA of *Mycobacterium smegmatis* is a target for triclosan. *Antimicrobial Agents and Chemotheraphy, 43*, 711-713.

[9] Russell, A.D., & Chopra, I. (1996). Understanding antibacterial action and resistance. 2nd edition, Ellis Horwood, Chichester, U.K.

[10] Hamilton, W.A. (1968). The mechanism of the bacteriostatic action of tetrachlorosalicylanilide: a membrane-active antibacterial compound. *Journal of General Microbiology*, 441-458.

[11] Huiyi, L., Shurong, L., & Tonghui, Z. (1999). Studies on *in vitro* metabolism of shikonin. *Phytotherapy Research, 13*, 236-238.

[12] Yoon, Y., Kim,Y., Lim, N., Jeon, W., & Sung, H.J. (1999). Shikonin, an ingredient of *Lithospermum erythrorhizon* induced apoptosis in HL60 human premyelocytic leukemia cell line. *Planta Medica, 65*, 532-535.

[13] Hisa, T., Kimura, Y., Takada, K., Suzuki, F., & Takigawa, M. (1998). Shikonin, an ingredient of *Lithospermum erythrorhizon*, inhibits angiogenesis in vivo and in vitro. *Anticancer Res., 18*(2A), 783-90.

[14] Papageorgiou, V.P., Assimopoulou, A.N., Couladouros, E.A., Hepworth, D., & Nicolaou, K.C. (1999). The chemistry and biology of alkannin, shikonin, and related naphthazarin natural products. *Angewandte Chemie International Edition, 38*, 270-300.

[15] Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564-582.