TEKSTİL VE MÜHENDİS

(Journal of Textiles and Engineer)

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Determination of Antimicrobial Activity of the Dyed Silk Fabrics with Some Natural Dyes

Bazı Doğal Boyalar Kullanılarak Boyanmış İpek Kumaşların Antimikrobiyal Aktivitesinin Belirlenmesi

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Online Erişime Açıldığı Tarih (Available online): 30 Mart 2015 (30 March 2015)

Bu makaleye atıf yapmak için (To cite this article):

Rezan ALKAN, Emine TORGAN, Canan AYDIN, Recep KARADAG (2015): Determination of Antimicrobial Activity of the Dyed Silk Fabrics with Some Natural Dyes, Tekstil ve Mühendis, 22: 97, 37-43.

For online version of the article: http://dx.doi.org/10.7216/130075992015229706

TMMOB Tekstil Mühendisleri Odası
UCTEA Chamber of Textile Engineers
Tekstil ve Mühendis
Journal of Textiles and Engineer

Yıl (Year) : 2015/1
Cilt (Vol) : 22
Sayı (No) : 97

Research Article / Araştırma Makalesi

DETERMINATION OF ANTIMICROBIAL ACTIVITY OF THE DYED SILK FABRICS WITH SOME NATURAL DYES

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ABSTRACT: In this study, silk fabric is dyed with natural indigo. Dyed silk fabric with natural indigo was cut in the 20x20 cm^2 size. Excluding a fabric, all fabrics were mordanted in the same percentage with alum metal ($KAl(SO_4)_2.12H_2O$). Then, silk fabrics for green color dyeing are dyed separately with weld (*Reseda luteola*), gall oak (*Quercus infectoria* Olivier) and together weld (*Reseda luteola*) and gall oak (*Quercus infectoria*) in different percentage. Antimicrobial functionality of the twenty seven silk fabrics is established. Tests were conducted against the *Staphylococcus aureus* ATCC 6538. The results of the counting test showed more reduction of survival *Staphylococcus aureus* in dark-colored fabric. The number of survival microorganism was determined by counting the colonies as colony-forming unit (CFU/ml) and reduction rate of bacteria was calculated. Coloring compounds and their percentages in the natural dyed silk fabrics are detected by HPLC-PDA (high performance liquid chromatography with diode array detection). Colour measurement is done of the dyed silk fabrics by CIEL*a*b* spectrophotometer.

Keywords: Antimicrobial testing, natural dyeing, *Staphylococcus aureus*, HPLC-PDA, colour measurement.

BAZI DOĞAL BOYALAR KULLANILARAK BOYANMIŞ İPEK KUMAŞLARIN ANTİMİKROBİYAL AKTİVİTESİNİN BELİRLENMESİ

ÖZET Bu çalışmada, ipek kumaş doğal indigo bitkisi ile boyanmıştır. Sonra 20x20 cm^2 boyutunda kesilmiştir. Bir kumaş hariç tüm kumaşlar aynı yüzdede şap ($KAl(SO_4)_2.12H_2O$) çözeltisi ile mordanlanmıştır. Yeşil renk boyama için kumaşlar önce ayrı ayrı muhabbet çiçeği (*Reseda luteola*) ve mazı gomalağı (*Quercus infectoria* Olivier) ile daha sonra iki bitki beraber farklı yüzdelerde kullanılarak boyama yapılmıştır. Antimikrobiyel test 27 adet ipek kumaş için uygulanmıştır. Testler *Staphylococcus aureus A*TCC 6538 e karşı yapılmıştır. Sayım sonuçları koyu renkli kumaşlarda *S.aureus'* un daha fazla azaldığını göstermiştir. Canlı mikroorganizma sayısı CFU/ml olarak koloni sayımı ile belirlenmiştir. Bakteri azalması tayin edilmiştir. Boyama yapılmış ipek kumaşlardaki renk bileşenleri ve onların yüzdeleri HPLC-PDA (yüksek performanslı sıvı kromatografisi) ile renk ölçümleri ise CIEL*a*b* spektorofotometresi yardımıyla belirlenmiştir.

Anahtar Kelimeler: Antimikrobiyal test, doğal boyama, *Staphylococcus aureus*, HPLC-PDA, renk ölçümü.

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DOI: *10.7216/130075992015229706, www.tekstilvemuhendis.org.tr*

1. INTRODUCTION

In recent years, natural dyes have attracted renewed attention because of their biodegradability, sustainable production and uncommon, soothing shades. Synthetic dyes are often economical and available in a wide variety of colours, but they (e.g. MAK III category 2 dyes, which belong to the carcinogenic dyes used in the textile industry [1]) may cause skin allergies and other harm to the human body, in addition to producing toxic waste. Natural dyes are obtained from renewable sources such as crops, insects and so forth, and they may decrease the dependence on petrochemical sources [2–4]. These considerations have led to the publication of several studies on natural dyes from a number of sources [5–11]. In former times, wool or silk fibres were always dyed with natural dyes extracted from plants or animals [12]. Compounds present in extracts obtained from the most widely used natural dyes belong to a few main classes: flavonoids (yellow dyes), anthraquinoids (red dyes), indigoids (purple and blue dyes) and tannins (brown and black dyes) [13,14].

Natural dyes can be obtained from plants, animals and minerals [15,16]. It is reported that many natural dyes can not only dye unique and natural shades, but can also provide functions to fabrics such as antibacterial activity, ultraviolet protection and insect repellency. These natural dyes have been successfully applied to natural fiber fabrics such as cotton, wool, silk and flax. However, limited availability and high-cost restricted the industrialization of many natural dyestuufs [16].

Natural dyes are reported as potent antimicrobial agents owing to the presence of a large amount of compounds such as antraquinones, flavonoids, tannins, naphtoquinones etc. which possess strong antimicrobial properties. Though a plethora of natural antimicrobial agents exist especially against common human pathogens however; very few studies have been reported in the literature regarding the antimicrobial properties on textile materials with respect to the human pathogenic strains [17].

Flavonoids and phenolic acids are ubiquitous bioactive compound found in plant foods and beverages. Flavonoids can be grouped in several structural classes including anthocyanins, flavones, flavan-3-ols, flavonols, and tannins. These flavonoid compounds share the same basic structure consisting of two aromatic rings joined in a chroman structure by a three-carbon unit: C_6-C_3-C_6 [18].

Several analytical techniques for the identification of natural dyes present in textiles have been applied, such as high performance liquid chromatography (HPLC), ultraviolet–visible (UV–vis) spectrophotometry, thin-layer chromatography, Raman spectroscopy, micro spectrofluorimetry and gas chromatography⁄ mass spectrometry. HPLC has become an important method for identification of natural dyes present in historical textiles, art objects, etc. [19-21]. In this study, the HPLC diode array detection (DAD) method was used for the separation and identification of flavonoid, tanin and indigoid dyes components present in silk fabrics dyed with indigo dyes (*Indigofera tinctoria* L. or *Isatis tinctoria* L.), weld (*Reseda luteola*) and gall oak (*Quercus infectoria* Olivier).

The aim of this study, antibacterial and antimicrobial functionallity were detected for silk fabrics dyed green colour according to historical recipe. Coloring compounds and their percentage in the natural dyed silk fabrics are detected by HPLC-PDA (high pressure liquid chromatography with diode array detection). Colour measurement is done of the dyed silk fabrics by CIEL*a*b* spectrophotometer.

2-MATERIAL AND METHODS

2.1 Dye plants and chemicals

Indigo dye (*Isatis tinctoria* L.), weld (*Reseda luteola*) and gall oak (*Quercus infectoria* Olivier) plants were provided from TCF (Turkish Cultural Foundation)-Armaggan company. The following dye standards were used as references: luteolin from Carl Roth (Germany), indigotin, indirubin and apigenin from Sigma-Aldrich and ellagic acid from Alfa asesar.

Alum [$KAl(SO_4)_2.12H_2O$], hydrochloric acid and methanol were obtained from Merck (Germany). Nutrient agar and nutrient broth were purchased from Oxoid. Culture *Staphylococcus aureus* ATCC 6538 was used for antibacterial evaluation.

2.2 Dyeing procedures for silk fabrics

In this study, silk fabric is dyed with natural indigo. Dyed silk fabric with natural indigo was cut in the 20x20 cm^2 size. Excluding a fabric, all fabrics were mordanted in the same percentage with alum metal. Then, silk fabrics for green color dyeing are dyed separately with weld (*Reseda luteola*), gall oak (*Quercus infectoria*) and together weld (*Reseda luteola*) and gall oak (*Quercus infectoria*) in different percentage. Natural dyeing process is shown in the Table 1.

Table 1. Dyeing procedures for silk fabrics.

No.	İndigo plant	Mordant (%)	Gall oak (%)	Weld (%)	İndigo Dyeing Temp. (^oC)	Mordanting Temp. (^oC)	Dyeing Temp. (^oC)	İndigo Dyeing Time (min.)	Mordanting Time (min.)	Dyeing Time (min.)
1	-	-	-	-	-	-	-	-	-	-
2	x	-	-	-	50	-	-	2	-	-
3	x	6	-	-	50	65	-	2	60	-
4	x	6	5	-	50	65	80	2	60	60
5	x	6	10	-	50	65	80	2	60	60
6	x	6	15	-	50	65	80	2	60	60
7	x	6	20	-	50	65	80	2	60	60
8	x	6	-	25	50	65	80	2	60	60
9	x	6	-	50	50	65	80	2	60	60
10	x	6	-	75	50	65	80	2	60	60
11	x	6	-	100	50	65	80	2	60	60
12	x	6	5	25	50	65	80	2	60	60
13	x	6	5	50	50	65	80	2	60	60
14	x	6	5	75	50	65	80	2	60	60
15	x	6	5	100	50	65	80	2	60	60
16	x	6	10	25	50	65	80	2	60	60
17	x	6	10	50	50	65	80	2	60	60
18	x	6	10	75	50	65	80	2	60	60
19	x	6	10	100	50	65	80	2	60	60
20	x	6	15	25	50	65	80	2	60	60
21	x	6	15	50	50	65	80	2	60	60
22	x	6	15	75	50	65	80	2	60	60
23	x	6	15	100	50	65	80	2	60	60
24	x	6	20	25	50	65	80	2	60	60
25	x	6	20	50	50	65	80	2	60	60
26	x	6	20	75	50	65	80	2	60	60
27	x	6	20	100	50	65	80	2	60	60

2.3 Determination of Antimicrobial Activity of Dyed Silk Fabrics

AATCC Test Method 100-1999 was used to determine the antimicrobial activity. The antimicrobial activity of fabrics against Staphylococcus aureus ATCC 6538,a pathogenic gram-positive bacterium, was used because the major cause of cross-infection in hospitals. The circular fabric specimens (4.80 ±0.1 cm) were placed in container and sterilized for 15 min at 121^0C. Staphylococcus aureus was grown in nutrient broth medium for 24 hr at 37 ± 1^0C. The inoculum was a nutrient broth culture containing $1x10^5$ (CFU/ml) of bacteria. An aliquot of 1000µliter bacterial suspensions were added to the center of 4.80 ±0.1 cm fabric and incubated for 24 hr at 37 ± 1^0C. Thefabric was resuspended in dilution medium, vigorously shaken 1 min prior to the dilution. Ten fold serial dilutions were made to all samples. A fixed volume of each dilution (100 µliter) was inoculated on nutrient agar plates and the plates were incubated at 37 ± 1^0C for 24 hr. Viable colonies of bacteria on the agar plate were counted and the percentage of reduction in the number of bacteria was calculated using Eq (1):

$$R\ (\%) = A\text{-}B/A \times 100$$

Where R is the percentage reduction of bacteria, A represents the number of bacteria colonies in the control (the untreated fabric), and B represents the number of bacteria colonies in the treated fabrics.

2.4 Colour Measurement of the Dyed Silk Fabrics

L*, a* and b* values for dyed silk fabrics were measured with Konica Minolta CM-2300d Software Spectra Magic NX (6500 K, 45^o). CIEL*a*b* graphs and L*, a* and b* values were shown in Table 5.

2.5 HPLC-PDA Analysis

2.5.1 Sample Preparation for HPLC Analysis of Dyed Silk Fabrics

The extraction of twenty seven samples were performed with a solution mixture of %37 HCl: MeOH: H_2O 2:1:1; v:v:v) for 8 minutes at 100 ^{o}C in open small tubes to extract dyestuffs. After cooling under running cold tap water, the solution was evaporated just to dryness in a water bath at 65 ^{o}C under a gently stream of nitrogen. The dry residue was dissolved in 200 µl of the mixture of MeOH:H_2O (2:1; v:v) was centrifuged at 4000 rpm for 10 min. 100 µl supernatant was injected into the HPLC apparatus.

2.5.2 HPLC Instrumentation

Chromatographic measurements were carried out using an Agilent 1200 series system (Agilent Technologies, Hewlett-Packard, Germany) including G1322A Degasser, G1311A Quat pump, G1329A autosample, G13166 TCC, and G1315D Diode Array Detector. PDA detection is performed by scanning from 191 to 799 nm with a resolution of 2 nm, and the chromatographic peaks were monitored at 255, 268, 276, 350, 491, 520, 580 and 620. Column: A Nova Pak C18 analytical column (39×150 mm, 4 µm, Part No WAT 086344, Waters) was used. Analytical and guard columns were maintained at 30°C and data station was the Agilent Chemstation. Two solvents were utilized for chromatographic separations of the hydrolysed samples. Solvent A: H_2O - 0.1% TFA and solvent B: CH_3CN- 0.1 % TFA. The flow rate was 0.5 mL/min. and following elution program was applied (Table 2).

Table 2. Gradient elution parameters for HPLC.

Time (min.)	Flow rate (ml/min)	H_2O-0,1% TFA (v/v)	CH_3CN-0,1% TFA (v/v)
0.0	0.5	95	5
1.0	0.5	95	5
20	0.5	70	30
25	0.5	40	60
28	0.5	40	60
33	0.5	5	95
35	0.5	5	95
40	0.5	95	5
45	0.5	95	5

3. RESULT AND EVALUATION

Twenty six silk fabrics are dyed with natural dyes that used dyes are natural indigo (*Indigofera tinctoria* L. or *Isatis tinctoria* L.), gall oak (*Quercus infectoria* Olivier) and weld (*Reseda luteola*) (Figure 1). Antibacterial and antimicrobial activity was analyzed of the dyed silk fabrics with natural dyes. Obtained

the best results are reported (Table 3). High Performance Liquid Chromatography (HPLC) using Diode-Array Detection (DAD) is ideally suited for identification of natural dyestuffs present in these materials [22, 23].

The standard dyestuffs used in the present study, such as ellagic acid, luteolin, apigenin, indigotin and indirubin were also chromatographically and spectrophotometrically (UV-Vis) characterized. In this study, peak height of the identified dyestuffs is shown in Table 4. Colorimetric values of silk fabrics are presented in Table 5.

Table 3. Antimicrobial activity of dyed silk fabrics.

Dyeing Code	Bacterial reduction (%)
1*	-
8	-
9	-
10	-
11	-
20	99,19
21	96,02
22	98,07
23	99,68
24	99,91
25	91,98
26	98,69
27	98,78

1*: not dyeing silk fabric.

a **b** **c**

Figure 1. Used natural dye sources in the dyeing. **a-** woad (*Isatis tinctoria* L.) **b-** gall oak (*Quercus infectoria* Olivier) **c-** weld (*Reseda luteola*). Photos by Prof. Dr. Recep Karadag.

Table 4. Peak height of the identified dyestuffs in the HPLC analysis.

Dyeing Code	Peak height of the identified dyestuffs (at 255 nm)				
	ellagic acid	luteolin	apigenin	indigotin	indirubin
8	-	21.4	1.1	-	3.2
9	-	51.4	1.5	-	3.8
10	-	56.5	1.8	-	4.1
11	-	98.2	3.8	-	2.5
20	489.8	16.0	1.9	-	-
21	651.2	67.2	-	-	2.7
22	514.7	31.9	4.7	-	1.9
23	554.5	53.3	2.3	-	2.7
24	633.9	29.1	-	1.1	2.0
25	279.4	32.7	5.1	-	3.1
26	761.3	66.6	-	-	2.9
27	717.6	87.0	6.5	-	2.0

Table 5. L*, a* and b* values of the dyed silk fabrics.

Dyeing Code	CIEL*a*b* Değerleri		
	L*	a*	b*
1	94.29	-0.05	5.17
2	65.32	-7.23	-15.94
3	67.24	-2.85	-10.93
4	66.76	-5.76	0.35
5	64.65	-5.91	8.29
6	63.03	-5.08	11.68
7	61.02	-6.64	11.67
8	66.82	-14.04	33.07
9	65.90	-14.28	44.29
10	67.87	-13.12	45.59
11	66.14	-12.43	53.54
12	64.07	-12.04	34.76
13	62.72	-12.30	39.21
14	62.40	-12.05	45.84
15	61.26	-11.34	45.35
16	59.48	-7.63	28.88
17	59.93	-8.63	33.20
18	60.44	-8.40	36.33
19	59.96	-9.45	37.96
20	61.79	-4.18	22.74
21	60.15	-4.25	26.86
22	60.61	-5.60	28.03
23	60.51	-5.59	30.10
24	61.63	-3.30	24.24
25	60.12	-4.41	25.68
26	60.55	-3.30	25.08
27	60.05	-3.42	26.24

4- CONCLUSION

Although the antibacterial properties of the apigenin dyestuffs in the literature, apigenin dyestuffs in the *Reseda luteola* plant used dyeing was detected to have low content. This also shows that has no effect to *Staphylococcus aureus* of the apigenin dyestuffs.

The results of the both HPLC and antibacterial analysis shows that antibacterial activity was not detected in the sample of 8-11 dyeing code. The reason for this, in these dyeings gall oak (*Quercus infectoria* Olivier) plant is not used that consist gallic acid, ellagic acid, etc.

Antibacterial activity was determined to increase in the dyeings belong to 20-27 dyeing code. In these dyeings, 15-20% gall oak (*Quercus infectoria* Olivier) plant was used. Stable antibacterial activity was determined by 20% to 15% of gall oak.

ACKNOWLEDGEMENT

This work was supported by the Turkish Cultural Foundation (TCF) Cultural Heritage Preservation and Natural Dyes Laboratory and Armaggan company are gratefully acknowledged.

(http://www.turkishculturalfoundation.org, http://www.tcfdatu.org, www.armaggan.com).

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