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Colorimetric Assay and Antibacterial Activity of Cotton, Silk, and Wool Fabrics Dyed with Peony, Pomegranate, Clove, *Coptis chinensis* and Gallnut Extracts

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Abstract: To investigate the antibacterial functionality of natural colorant extracts, five kinds of natural dyeing aqueous solutions were obtained by extraction from peony, pomegranate, clove, *Coptis chinensis* and gallnut using water at 90 °C for 90 min with a liquor ratio (solid natural colorant material/water, weight ratio) of 1:10. The colorimetric assay and antibacterial activity of cotton, silk, and wool fabrics dyed with these natural colorant extracts were examined. It was found that these properties were significantly dependent on the structure of colorant and the kind of fabrics. The hues (H) of all fabrics dyed with these natural colorants were in the range of 6.05YR -1.95Y. The order of value (V) was wool, silk and cotton. The chroma (C) of all samples was found to be at very low levels indicating the natural tone. All the fabrics dyed with the five natural colorants (peony, pomegranate, clove, *Coptis chinensis* and gallnut) extracts displayed excellent antibacterial activity (reduction rate: 96.8 - 99.9%) against *Staphylococcus aureus*. However, in the case of *Klebsiella pneumoniae*, the antibacterial activity was found to depend on the kind of natural colorant extract used.

Keywords: Antibacterial activity; Natural colorants; Colorimetric assay.

1. Introduction

Recently there has been a revival of interest in the use of natural dyes in textile coloration. This is a result of the stringent environmental standards imposed by many countries in response to the toxic and allergic reactions associated with the use of synthetic dyes. A widespread interest has emerged in the dyeing of textile fibres using natural colorants, on account of their high compatibility with environment, softer colour shades, naturalness, lower toxicity and antibacterial/anti-allergic/deodorizing/anti-cancer properties, harmonising natural shades or just the novelty [1-6].

It is well known that problems in dyeing with natural dyes are the low exhaustion of natural colorants and the poor fastness of dyed fabrics. Attempts to overcome these problems have been mainly focused on the use of metallic salts as mordants, which are traditionally used to improve fastness properties or exhaustion and to develop different shades with the same dye [7-12]. However, most synthetic mordants are toxic heavy metal ions, which are also pollutants. Reports on dyeing properties using natural colorants without mordants are currently not available in the open literature, which makes achievement high exhaustion and good fastness using natural colorants without mordants all the more significant.

In our previous studies, the dyeability, fastness and deodorizing properties of cotton, silk, and wool fabrics dyed with some natural colorants (sappan wood, black tea, peony, clove, gardenia, coffee sludge, *Cassia tora*. L., and pomegranate) without mordant were investigated [13-15]. These properties were found to be closely dependent on the extract concentration, colorant structure and fabric type. The fastness (light, water, and perspiration fastness: 2nd - 5th grades) and the deodorizing performance (34-99%) were found to be related to the K/S and the combination between fabrics and natural colorant extracts. It was found that the use of some natural colorants notably enhanced the deodorizing performance.

As living standards have improved, people have become extremely concerned about health and hygiene. An increasing volume of literature demonstrates the survival and growth of harmful microorganisms (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Neisseria*, *Bacillus subtilis*, *Diphtheroid bacilli*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Klebsiella*, *Enterobacter aerogenes*, *Citrobacter* and *Candida albicans*) in textiles and the health risks of their dissemination [16]. Major antibacterial agents for textiles include metals, metal based compounds, phenolic compounds and quaternary ammonium salts, etc. which all have toxicity and environmental issues. It has become increasingly important for antibacterial agents to meet environmental and low toxicity criteria, while retaining their functionality. Therefore, it is vital to research and develop eco-friendly antibacterial agents extracted from plants/animals for textile applications. The effect of various plants on bacteria has been studied by a number of researchers [17-19]. However, despite the fact that there are many natural antibacterial agents, few studies in the open literature have explored their antibacterial activity on textile materials.

This study investigated the antibacterial functionality of cotton, silk, and wool fabrics dyed using five kinds of natural aqueous dyeing solutions obtained by extraction from peony, pomegranate, clove, *Coptis chinensis*, and gallnut. *Staphylococcus aureus* and *Klebsiella pneumoniae*, the micro-organisms typically known to grow on textiles, were used in this study. The colorimetric assay and antibacterial

activity of cotton, silk, and wool fabrics dyed with above natural colorant extracts without mordants were also examined in this work.

2. Results and Discussion

2.1. Confirmation of components of various natural colorant extracts

Figure 1 shows the IR spectra obtained for the peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts.

Peony extract (a) showed characteristic peaks corresponding to the phenol at $3,200\text{ cm}^{-1}$ and $1,238\text{ cm}^{-1}$, phenyl CH stretch at $2,903\text{ cm}^{-1}$, ring stretch (benzene ring in aromatic compounds) at $1,636\text{ cm}^{-1}$ and the C=O stretching at $1,419$, $1,086$, and $1,040\text{ cm}^{-1}$ were observed, potentially indicating the presence of paeonol/paeonoside/paeonolide/paeoniflorin in the peony extract.

Pomegranate extract (b) had characteristic peaks corresponding to the OH at $3,384\text{ cm}^{-1}$, CH anti-symmetric and symmetric stretch at $2,926\text{ cm}^{-1}$, C=O stretch at $1,732\text{ cm}^{-1}$, C=C stretch at $1,603\text{ cm}^{-1}$, C-O stretch at $1,071\text{ cm}^{-1}$ were observed. This indicated the presence of ellagic acid in this extract.

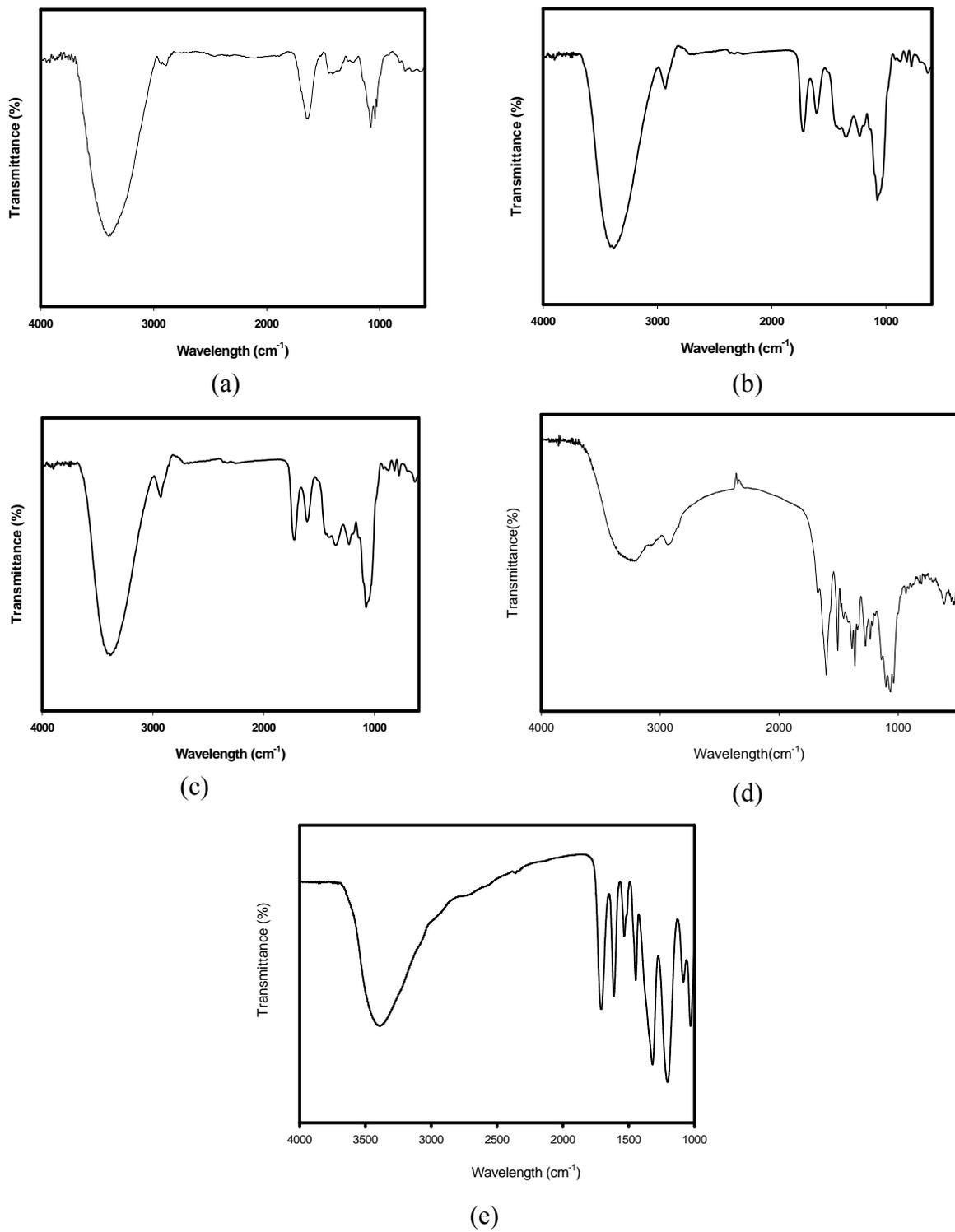
Clove extract (c) has the characteristic peaks corresponding to the vinyl =CH stretching at $2,931\text{ cm}^{-1}$, the overtone of vinyl CH₂ out-of-plane wagging and C=O stretch at $1,724\text{ cm}^{-1}$, ring stretch (benzene ring in aromatic compounds) at $1,608\text{ cm}^{-1}$, CH₃ antisymmetric deformation at $1,447\text{ cm}^{-1}$ and $1,359\text{ cm}^{-1}$, phenol at $1,220\text{ cm}^{-1}$, C-O stretch at $1,044\text{ cm}^{-1}$ indicating the presence of eugenol in clove extract.

Coptis chinensis extract (d) has the characteristic peaks corresponding to the OH stretch at $3,300\text{ cm}^{-1}$, NH deformation at $1,506\text{ cm}^{-1}$, CH₃ deformation at $1,387\text{ cm}^{-1}$, -CH₃ sym deformation at $1,362\text{ cm}^{-1}$, C-O-C antisym stretch at $1,233\text{ cm}^{-1}$, and C-O stretch at $1,075\text{ cm}^{-1}$. This indicated the presence of berberine, coptisine, worenine in *Coptis chinensis*.

Gallnut extract (e) displayed characteristic peaks corresponding to the OH stretch at $3,382\text{ cm}^{-1}$, C=O stretch at $1,709\text{ cm}^{-1}$, ring stretch at $1,612\text{ cm}^{-1}$, C-O-C antisym stretch at $1,362\text{ cm}^{-1}$, C-O stretch at $1,030\text{ cm}^{-1}$. This indicated the presence of penta-*m*-digalloyl- β -glucose, gallic acid in gallnut.

Natural colorant extracts are composed of main component and many minor components yet to be characterized fully. The FTIR characterization presented herein can potentially identify components present in the extracts for future studies to determine the source of the extract antibacterial activity. A detailed analysis of each major and minor component will require further characterization by confirmatory chromatographic and mass spectrometric techniques.

Figure 1. Infrared spectra of extracted natural colorants: peony (a), pomegranate (b), clove (c), *Coptis chinensis* (d) and gallnut (e).



2.2. Concentrations of extracted natural colorants

From the results of our previous study, the optimum liquor ratio was found to be 1:10 [15]. Therefore, all natural colorants were extracted at 1:10 liquor ratio in this study. The concentrations of peony, pomegranate, clove, *Coptis chinensis*, and gallnut extracts were found to be 1.71, 0.96, 0.92, 1.92, and 6.41g/100 mL, respectively. The concentration of extract increased in the order of clove < pomegranate < peony < *Coptis chinensis* < gallnut.

2.3. Colorimetric assay

In this study, the dyeing time and temperature were fixed at 60 min and 80 °C, respectively. It was because the preliminary experiment found that the optimum dyeing time and temperature at a fixed liquor ratio (1: 10) were 60 min and 80 °C respectively [15].

Table 1. Colorimetric data of fabrics dyed with peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts.

Fabric	Natural colorant	K/S	L*	a*	b*	H	V/C
Cotton	Control	0.08	88.97	-0.25	1.10		
	Peony	0.54	84.47	0.94	5.92	9.48YR	8.34/0.91
	Pomegranate	1.18	86.51	0.09	11.22	1.84Y	8.55/1.53
	Clove	1.70	80.08	2.40	16.92	0.31Y	7.88/2.61
	<i>Coptis chinensis</i>	2.08	76.15	1.81	24.96	1.94Y	7.47/3.73
	Gallnut	0.52	88.48	-0.03	8.15	1.95Y	8.75/1.09
Silk	Control	0.11	86.87	-0.21	1.10		
	Peony	1.76	75.13	3.55	7.92	6.05YR	7.36/1.56
	Pomegranate	3.18	80.39	1.39	17.13	1.22Y	7.91/2.54
	Clove	7.00	64.28	7.38	28.66	9.54YR	6.25/4.78
	<i>Coptis chinensis</i>	7.32	64.79	9.39	51.44	0.63Y	6.30/8.11
	Gallnut	1.10	84.74	1.5	7.54	8.87YR	8.36/1.20
Wool	Control	0.27	86.38	-1.23	5.00	-	-
	Peony	4.26	66.77	4.40	15.91	8.97YR	6.50/2.67
	Pomegranate	5.97	73.97	3.43	33.09	1.72Y	7.24/5.04
	Clove	10.46	54.40	11.16	35.03	8.80YR	5.26/5.92
	<i>Coptis chinensis</i>	11.01	56.33	10.50	48.57	0.30Y	5.45/7.71
	Gallnut	3.34	72.75	3.38	17.67	9.98YR	7.11/2.81

Table 1 and Figure 2 show colorimetric parameter (L^* , a^* , b^* , H and V/C) of fabrics (cotton, silk, and wool) dyed with peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts. The values

quoted are the averages of five measurements. Generally, the dyeing affinity of textile materials is dependent on the content and type/polarity of functional groups of fibers. It is well known that the number of functional group in wool is larger than that of silk, and polarity of protein fibers is higher than that of cellulose fibers [20].

The color hues of cotton dyed with peony, pomegranate, clove, *Coptis chinensis* and gallnut were found to be 9.48YR, 1.84Y, 0.31Y, 1.94Y, and 1.95Y, respectively. The color hues of silk dyed with these natural colorants were 6.05YR, 1.22Y, 9.54YR, 0.63Y, and 8.87YR, respectively. The values of wool were 8.97YR, 1.72Y, 8.80YR, 0.30Y, and 9.98YR, respectively. In this study, various color characters of dyed fabrics were characterized using coordinate a^* (redness) and b^* (yellowness) as shown in Figure 2. Wool showed the highest a^* and b^* values for all colorants (peony, pomegranate, clove, *Coptis chinensis* and gallnut), followed by silk and cotton, indicating that the increase of redness and yellowness was in the order of wool > silk > cotton (see Table 1 and Figure 2). Various apparent colors of fabrics (cotton silk and wool fabrics) dyed with five kinds of natural colorants are shown in Figure 3. It was found that the color of dyed fabrics was dependent on the kinds of natural colorants and fibers. Various elegant colors were obtained using natural colorant extracts such as peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts (see Figure 3).

Figure 2. Various colors of dyed fabrics on coordinate a^* (greenness-redness) and b^* (blueness-yellowness).

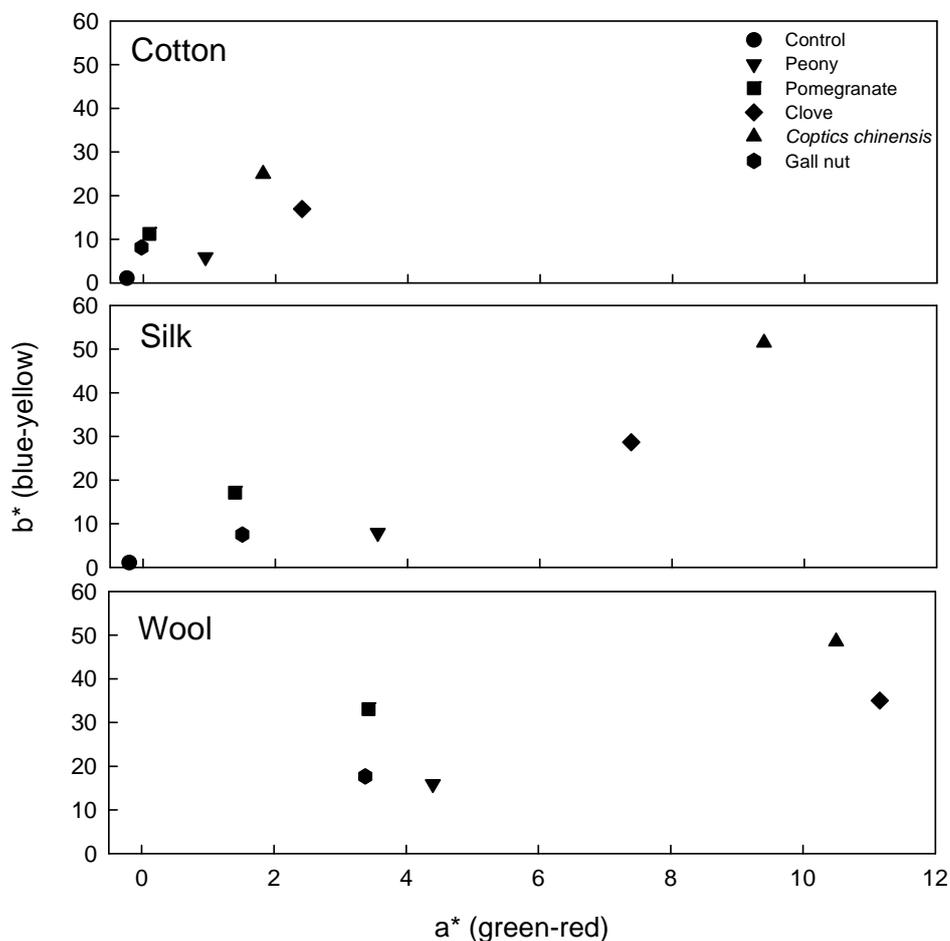
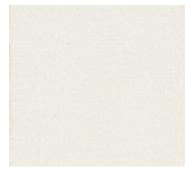
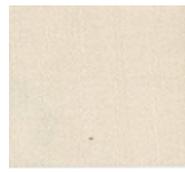


Figure 3. Various colors of dyed fabrics (cotton, silk, and wool fabrics) using peony, pomegranate, clove, *Coptis chinensis* and Gallnut.

	Peony	Pomegranate	Clove	<i>Coptis chinensis</i>	Gallnut
Cotton					
Silk					
Wool					

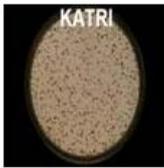
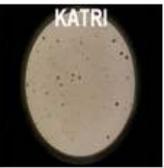
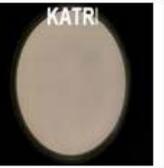
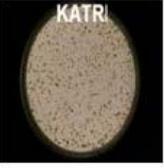
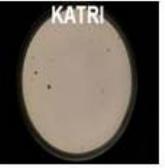
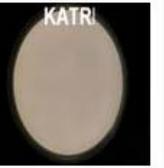
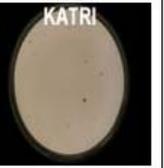
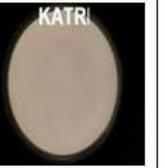
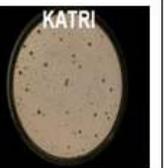
2.4. Antibacterial activity

Figure 4 shows the micrographs of antibacterial activity of control fabrics and dyed fabrics (cotton, silk, and wool fabrics dyed with peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts) against *Staphylococcus aureus* and *Klebsiella pneumonia*. The bacteriostatic reduction rates of control fabric and dyed fabrics against *Staphylococcus aureus* and *Klebsiella pneumonia* are given in Table 2. All the fabrics dyed with the four kinds of natural colorants (pomegranate, clove, *Coptis chinensis* and gallnut) extracts displayed outstanding antibacterial activities against *Staphylococcus aureus* (reduction rate: 96.8 - 99.9%) and *Klebsiella pneumonia* (95.7- 99.9%). All the fabrics dyed with peony extract showed excellent antibacterial activity (reduction rate: 98.8 - 99.7%) against *Staphylococcus aureus*, however, peony did not display antibacterial activity against *Klebsiella pneumonia*.

The antibacterial activity of pomegranate extract might be attributable to ellagic acid and tannin components. The antibacterial activity of *Coptis chinensis* extract might be due to the berberine which is known to possess good antibacterial activity. In the case of peony extract, although there was no special component which is known to show good antibacterial activity, the antibacterial activity of peony extract might be due to unknown minor components such as tannins and steroids. In the case of clove, the antibacterial activity might be creditable mainly to the phenol component in eugenol. Natural colorant extracts are composed of main component and many unknown components. Therefore, it is very difficult to identify the exact components that have antibacterial activity against microorganisms. More detailed studies should be made.

Figure 4. The micrographs of antibacterial activity of control fabrics and dyed fabrics using various natural colorants against (a) *Staphylococcus aureus* and (b) *Klebsiella pneumoniae*.

(a)

	Control	Peony	Pomegranate	Clove	<i>Coptis chinensis</i>	Gallnut
Cotton						
Silk						
Wool						

(b)

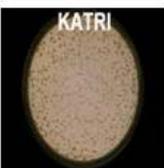
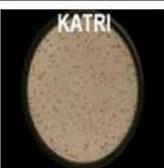
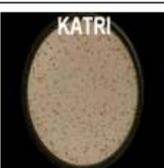
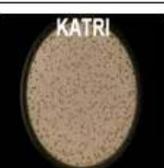
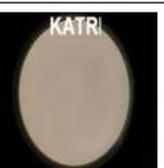
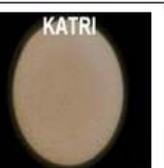
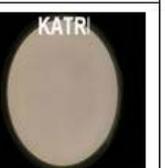
	Control	Peony	Pomegranate	Clove	<i>Coptis chinensis</i>	Gallnut
Cotton						
Silk						
Wool						

Table 2. The bacteriostatic reduction rates (%) of cotton, silk, and wool fabrics dyed with peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Natural colorant	Cotton		Silk		Wool	
	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
Pomegranate	99.9	95.8	99.8	99.5	99.9	99.8
Clove	99.9	99.9	99.9	99.9	99.9	99.9
Peony	98.8	0.0	99.7	0.0	99.4	0.0
<i>Coptis chinensis</i>	99.9	99.9	99.9	99.9	96.8	95.7
Gallnut	99.9	99.9	99.9	99.9	99.9	99.9

3. Experimental Section

3.1. Materials

Cotton, silk and wool fabrics (Standard Adjacent Fabrics for Staining of Fastness Test: KS K 0905) were used. The characteristics of these fabrics are shown in Table 3. Peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts were used as natural colorants.

Table 3. Characteristics of fabric samples.

	Cotton	Silk	Wool
Thickness ^a (mm)	0.27	0.10	0.33
Counts (Tex)	Warp	2.3/2	15.6/2
	Weft	2.3	15.6/2
Density (threads/cm)	Warp	38	18
	Weft	55	21
Weight (g/m ²)	115	26	125
<i>K/S</i>	0.08	0.11	0.27
<i>L*</i>	88.97	86.87	86.38
<i>a*</i>	-0.25	-0.21	-1.23
<i>b*</i>	1.10	1.03	5.00
WI	68.51	64.34	43.65
YI	1.97	1.93	8.69
BI	73.08	68.88	64.76

Weave: plain

^aThickness were measured under 1 kpa pressure

WI: Whiteness Index, 10deg./D65/Ganz

YI: Yellowness Index, 2deg./C/ASTM D1925

BI: Brightness Index, 2deg./C/TAPP1452/ISO2470

3.2. Extraction / Dyeing

Extraction: Five kinds of dyeing solutions were extracted from peony, pomegranate, clove, *Coptis chinensis* and gallnut using water at 90 °C for 90 min. at a fixed liquor ratio (solid natural colorant/water ratio) of 1:10.

Dyeing: All dyeing was carried out using a 1:100 bath ratio at 80 °C for 60 min by exhaustion method.

3.3. Characterization

To confirm the structure of natural colorants, FTIR spectrometer (Impact 400D, Nicolet, Madison, WI) was used to measure the infrared spectra of extract solution in the wavenumber of 400-4000 cm⁻¹ at room temperature. For each IR spectrometer samples 32 scans at 4 cm⁻¹ resolution was collected in the transmittance mode.

The reflectance values and the corresponding CIE L^* , a^* , b^* , H V/C, and color strength (K/S) values for the dyed samples were measured using a CCM (Gretag Macbeth Color-Eye 7000A, USA) interfaced to a digital PC under illuminant D₆₅, with a 10° standard observer. K/S was calculated from the reflectance values using the Kubelka-Munk equation as follows:

$$K/S = (1-R)^2/2R - (1-R_0)^2/2R_0$$

where R is the reflectance of the colored fabric, R_0 is the reflectance of the uncolored fabric, and K/S is the ratio of the absorption coefficient (K) to scattering coefficient (S): the higher the value, the greater the colour strength.

The antibacterial activity of the cotton, silk, and wool fabrics dyed with peony, pomegranate, clove, *Coptis chinensis* and gallnut against *Staphylococcus aureus* ATCC 6538 (gram positive) and *Klebsiella pneumoniae* ATCC 4352 (gram-negative) according to modified KSK 0693-2006 (Assessment of antibacterial activity). The concentrations of the cultures were adjusted using spectrophotometer(λ 660 nm) to 1.3×10^5 colony forming unit (CFU) per mL. The bacteriostatic reduction rate was estimated by the standard equation: Reduction (%) = [(A-B) / A] x 100, where, A and B is the bacteria colonies of untreated and treated fabrics, respectively.

4. Conclusions

Five kinds of natural dyeing solutions were obtained by extraction from peony, pomegranate, clove, *Coptis chinensis* and gallnut using water at 90 °C for 90 min with a liquor ratio of 1:10. The colorimetric assay and antibacterial activity of cotton, silk, and wool fabrics dyed with these natural colorant extracts were examined. It was found that these properties were closely dependent on the structure of colorant and the kind of fabrics. The hues of all fabrics dyed with these natural colorants were in the range of 6.05YR – 1.95Y (yellow red – yellow color). The order of V value was wool, silk and cotton indicating that the content of natural colorant in fiber increased in the order of wool > silk > cotton. The chroma (C) of all fabrics dyed natural colorant extracts obtained in this study was found to be very low values indicating their natural tones. All the fabrics dyed with the five kinds of natural colorants (peony, pomegranate, clove, *Coptis chinensis* and gallnut extracts) displayed excellent

antibacterial activity (reduction rate: 96.8 - 99.9%) against *Staphylococcus aureus*. All the fabrics dyed with pomegranate, clove, *Coptis chinensis* and gallnut extracts showed outstanding antibacterial activity (reduction rate: 95.7 - 99.9%) against *Klebsiella pneumoniae*, however, peony did not display antibacterial activity. These results clearly demonstrate that utilizing extracted natural colorants as dyeing materials significantly facilitate obtaining quality antibacterial fabrics having various natural colors.

References

1. Hill, D.J. Is There a Future for Natural Dyes? *Rev. Prog. Coloration* **1997**, *27*, 18-25.
2. Bechtold, T.; Turcanu, A.; Ganglberger, E.; Geissler, S. Natural Dyes in Modern Textile Dyehouse-How to Combine Experiences of Two Centuries to Meet the Demands of the Future? *J. Cleaner Production* **2003**, *11*, 499-509.
3. Kenneth, M.K. Color Characteristics of Traditional Vegetable Dyeing. *Text. Res. J.* **1973**, *43*, 404-407.
4. Montazer, M.; Parvinzadeh, M. Dyeing of Wool with Marigold and its Properties. *Fiber. Polym.* **2007**, *8*, 181-185.
5. Popoola, A.V. Dyeability of Cellulose Fibers using Dyestuff from African Rosewood (*Pterocarpus erinaceus*). *J. Appl. Polym. Sci.* **2000**, *77*, 746-751.
6. Angelini, L.G.; Pistelli, L.; Belloni, P.; Bertoli, A.; Panconesi, S. *Rubia tinctorum* a Source of Natural Dyes. *Ind. Crops Products* **1997**, *6*, 303-311.
7. Hwang, E.K.; Kim, M.S.; Lee, D.S.; Kim, K.B. Color Development of Natural Dyes with Some Mordants. *J. Kor. Fiber Soc.* **1998**, *35*, 490-497.
8. Shin, Y.S; Choi, H. Characteristics and Dyeing Properties of Green Tea Colorants (Part 2). *J. Kor. Soc. Cloth. Textil.* **1999**, *23*, 385-390.
9. Cho, K.R. Studies on the Natural dye (11). *J. Kor. Soc. Dyers Finishers* **1999**, *11*, 39-49.
10. Cristea, D.; Vilarem, G. Improving Light Fastness of Natural Dyes on Cotton Yarn. *Dyes Pigments* **2006**, *70*, 238-245.
11. Lee, Y.H.; Hwang, E.K.; Kim, H.D. Dyeing and Fastness of Silk and Cotton Fabrics Dyed with Cherry Extract. *J. Kor. Soc. Dyers Finishers* **2000**, *12*, 53-59.
12. Lee, Y.H.; Kim, H.D. Dyeing Properties and Colour Fastness of Cotton and Silk Fabrics Dyed with Cassia tora L. Extract. *Fibers and Polymers* **2004**, *5*, 303-308.
13. Lee, Y.H. Dyeing, Fastness, and Deodorizing Properties of Cotton, Silk, and Wool Fabrics Dyed with Coffee Sludge (*Coffea Arabica L.*) Extract. *J. App. Polym. Sci.* **2007**, *103*, 251-257.
14. Hwang, E.K.; Lee, Y.H.; Kim, H.D. Dyeing and Deodorizing Properties of Cotton, Silk, and Wool Fabrics Dyed with Various Natural Colorants. *J. Korean Soc. Dyers Finishers* **2007**, *19*, 278-286.
15. Hwang, E.K.; Lee, Y.H.; Kim, H.D. Dyeing, Fastness, and Deodorizing Properties of Cotton, Silk, and Wool Fabrics Dyed with Gardenia, Coffee sludge, *Cassia tora. L.*, and Pomegranate Extracts. *Fiber. Polym.* **2008**, *9*, 334-340.
16. Baumgartner, J.N.; Yang, C.Z. Physical Property Analysis and Bacterial Adhesion on a Series of Phosphonated Polyurethanes. *Biomaterials* **1997**, *18*, 831-837.

17. Liolios, C.; Laouer, H.; Boulaacheb, N.; Gortzi, O.; Ioanna, C. Chemical Composition and Antimicrobial Activity of the Essential Oil of Algerian Phlomis Bovei De Noe subsp. bovei . *Molecules* **2007**, *12*, 772-781.
18. Pereira, A.P. Phenolic Compounds and Antimicrobial Activity of Olive (*Olea europaea* L.Cv.Cobrancosa) Leaves. *Molecules* **2007**, *12*, 1153-1162.
19. Jasso de Rodriguez, D.; Hernandez-Castillo, D.; Angulo-Sanchez, J.L.; Rodriguez-Garcia, R.; Villarreal Quintanilla, J.A.; Lira-Saldivar, R.H. Antifungal activity in vitro of *Flourensia* spp. Extracts on *Alternaria* sp., *Rhizoctonia solani*, and *Fusarium oxysporum*. *Ind. Crops Products* **2007**, *25*, 111-116.
20. Vickerstaff, T. *The Physical Chemistry of Dyeing*; Oliver and Boyd: London, 1954; p. 346.

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