Research Article

Green Dyeing and Antibacterial Treatment of Hemp Fabrics Using *Punica granatum* Peel Extracts

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In this study, the dyeing performance and antibacterial properties of pomegranate (*Punica granatum*) peel extracts were investigated, when applied to hemp fabric. A tannin-rich natural dye was extracted using water at 100°C. As the antibacterial agent was sensitive to temperature, it was extracted using Soxhlet apparatus with methanol at 55°C. The color strength (*K/S*) values were investigated and found to increase in line with the dye concentration, dyeing time, and temperature. Optimal dyeing of the hemp fabric was achieved when applying 6% *w/v* of extract at 80°C for 60 min. This produced a yellowish-brown appearance. The dyed fabrics were rated as good to excellent for color fastness against washing, water, sea water, and perspiration. The dyed fabrics were next finished with the antibacterial agent extracted from the peel and tested for activity against *Staphylococcus aureus* (Gram positive) and *Klebsiella pneumoniae* (Gram negative), using the AATCC-100 (percentage reduction) test. The fabric was demonstrated to be 99.99% effective against *Staphylococcus aureus*, and this was conserved across 20 wash cycles.

1. Introduction

Increasing concern about the environment and health has encouraged researchers to look for ecofriendly resources. The natural materials obtained from plants, insects, and animals are renewable and sustainable bioresources with minimum environmental impact [1–3]. One of the most important justifications for using natural products is that they maintain the ecological balance by avoiding environmental impacts in production [4, 5]. Natural products are used not only as ingredients in food and cosmetics but also in textile applications. Natural dyes are extracted from many parts of the plant, including the bark, leaf, root, fruit or seed, and flower. Most natural dyes contain coloring materials such as tannin and are also inherently antimicrobial. They

consequently have medicinal applications as antimicrobials, antiallergens, or deodorants [6–12].

Pomegranate is the fruit of the deciduous shrub or small tree *Punica granatum*. Its peel has been used for traditional coloration of textiles since ancient times. Pomegranate peel is a rich source of tannins, phenolics, and alkaloids. Nine phenolic groups are present as major components [3, 13, 14]. There is also a considerable amount of tannin, making up approximately 20%, of the pomegranate peel [15]. Tannin can attach with textile substrates such as proteins (e.g., wool and silk) and cellulose fibers through a metallic mordant, as shown in Figure 1 [16]. Tannin can form hydrogen bonds between the phenolic hydroxyl groups of tannins and the hydroxyl group of the fabric. The extract of pomegranate peel has been used in dyeing and bactericidal treatment of cotton

FIGURE 1: Bonding of cellulose with tannin through metallic mordant [16].

fabrics. A wide range of shades, including yellow, brown, and black, and good fastness properties can be achieved by using copper and iron mordants. However, these are toxic [17–20].

Although the most popular cellulosic fabric is cotton, the climate of Thailand and many other tropical countries is unsuitable for its commercial production. Offering sustainability and economic value, hemp (*Cannabis sativa*) has been designated as an industrial crop in Thailand and can be cultivated in the northern region. It can be used to produce fabrics at lower cost than synthetic polymers [20, 21]. Hemp fibers comprise cellulose, hemicellulose, pectin, lignin, ester wax, water-soluble matter, and a small amount of ash. The presence of cellulosic substances in the raw hemp confers characteristics such as good water absorbency, comfort, and stability [22]. However, the water absorbency also encourages bacterial and fungal growth, resulting in odor and staining [23, 24]. Most research has focused on applying plant extract to fabrics such as cotton, silk, and wool, but few studies have investigated its use with hemp fabric [20, 25–27]. To encourage the use of hemp fabric in high-added-value products, the color yield and color fastness must be improved. The goal of this study was to apply the natural pomegranate extracts to hemp fabrics to improve their dyeability and antibacterial properties.

2. Materials and Methods

2.1. Materials. Plain weave hemp fabric with mass per unit area of 276 g/m² and warp and weft yarn counts of Ne 53 and Ne 62, respectively, were purchased from a local store in Thailand. The pomegranate peels were separated from other parts after the juice was extracted and then washed and dried at 60°C for 24 h and ground to obtain a powder.

Standard soap was purchased from SDC enterprises (UK) and aluminum potassium sulfate dodecahydrate (AlK(SO₄)₂·12H₂O) for fabric pretreatment was purchased from Ajax Finechem (Australia). Deionized water was used throughout the study. Methanol for the antibacterial agent extraction was purchased from Daejung (South Korea).

2.2. Preparation of Pomegranate Peel Extracts. The powdered pomegranate peel was soaked in deionized water for 24 h and heated at 100°C for 1 h at a 1 : 10 ratio of peel to water. The mixture was filtered and then spray dried at 150°C to yield a dye extract in the form of a brown powder. Antibacterial extract was produced by Soxhlet apparatus using methanol as solvent at a 1 : 10 ratio of peel to methanol. The temperature was controlled at 55°C. Solvent was removed by rotary evaporator, yielding a dark brown residue.

2.3. Dyeing and Antibacterial Finishing. In this section, we followed the method of Inprasit et al. [28]. Square hemp fabric samples (5 × 5 cm) were pretreated with 5% w/v standard soap at 100°C for 30 min and rinsed with deionized water. The premordanting method was used with a fixed concentration of 2% w/v AlK(SO₄)₂ to fix the dye onto the hemp fabrics by padding at a nip pressure of 2.5 kg/cm². Dyeing was performed using an IR dyeing machine (Starlet DL-6000). The liquor ratio was fixed at 1 : 30. The dye concentration, dyeing time, and dyeing temperature were varied to evaluate their effects on the dyeing process. The optimal dyeing condition was applied to all fabrics in the next step, in which antibacterial extract at a 1 : 2 ratio of extract to methanol was finished on the fabrics by padding at a nip pressure of 2.5 kg/cm², followed by air drying.

2.4. Color Measurement. The tests followed the same method [28]. The color of the dyed fabrics was evaluated using a spectrophotometer (Gretag-Macbeth color i5) with the CIELab color system (L^* , a^* , and b^*). L^* measures brightness, with 0 representing black and 100 representing white. a^* is the red-green coordinate (-ve = green and +ve = red), while b^* is the yellow-blue coordinate (-ve = blue and +ve = yellow). The K/S value corresponds to the color strength and is a measure of the amount of dye present in the fabric. The K/S value was calculated using the Kubelka–Munk equation:

$$\frac{K}{S} = \frac{(1 - R)^2}{R}, \quad (1)$$

where K is the absorption coefficient, R represents the reflectance of the sample, and S corresponds to the scattering coefficient. When the scattering from the dye is negligible, the K/S value is a linear function reflecting the amount of dye present in the sample [29, 30].

2.5. Color Fastness Testing. Color fastness of the dyed hemp fabric was tested using the AATCC standard. Color fastness to fresh water was tested using AATCC-107 and to sea water using AATCC-106. Dry and wet crocking tests were performed on the dyed fabrics following AATCC-8. Testing of color fastness to washing was conducted following ISO105-C01. Color fastness to acid and alkaline artificial perspiration was rated using ISO105-E04.

2.6. Antibacterial Testing. Quantitative determination of antibacterial activity on the dyed and antibacterial finished hemp fabrics against *Staphylococcus aureus* (a Gram-positive bacterium) and *Klebsiella pneumonia* (a Gram-negative bacterium) was performed using the percentage reduction method of AATCC-100. The activity of all treated fabrics was evaluated by the reduction in the number of bacterial colonies compared with the untreated fabric after incubation ($37 \pm 1^\circ\text{C}$, 24 h). The percentage reduction was calculated using Equation (2):

$$\text{Percentage reduction (R)} = \frac{(B - A)}{B} \times 100, \quad (2)$$

where R is the percentage reduction in bacterial colonies, A is the number of bacterial colonies recovered from the inoculated treated test specimen swatches incubated for 24 h contact period, and B is the number of bacterial colonies recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at “0” contact time).

Durability of antibacterial activity to washing was investigated using ISO105-C01. The experiment was conducted for 5, 10, 15, and 20 wash cycles of wash, and antibacterial activity was tested as above.

2.7. Characterization. The surface morphology and the presence of dye and antibacterial molecules in all hemp fabrics were examined using scanning electron microscopy (JEOL-JSM-IT200) with an acceleration voltage of 5 kV. All samples were coated with gold before analysis. FTIR (PerkinElmer,

Spectrum GX) was used to investigate the presence of pomegranate extract and its attachment onto the hemp fabrics.

3. Results and Discussion

3.1. Dyeing Conditions. The color properties of the dyed hemp fabrics were investigated using spectrophotometry in the wavelength range 380–750 nm. K/S is a measure of the color strength or intensity and is a linear function reflecting the amount of dye present in the fabric. In this work, the K/S had the highest value when the maximum absorbance was at 400–410 nm. Because the dye concentration, dyeing time, and temperature were all shown to affect the K/S value, three experiments were conducted. First, the dye concentration was varied at 2, 4, 6, 8, and 10% w/v with a fixed the temperature of 60°C for 60 min. Color measurement was then conducted. As shown in Figure 2(a), the K/S value increased as the dye concentration increased, indicating a higher number of dye molecules on the fabric surface. However, no significant increase in K/S was found between 6 and 10% w/v . This suggested a 6% w/v maximum dye concentration for this fabric. Second, the dyeing time was set at 30, 45, 60, 75, 90, 105, and 120 min with a fixed dye concentration 6% w/v and temperature at 60°C . Extending the dyeing time increased the K/S , until a constant value was reached of 60 min (Figure 2(b)). This implied that, after equilibrium was achieved, the hemp fabrics absorbed the dye more slowly. Pisitsak et al. [30] proposed that the prolonged heating may increase fiber decomposition, and therefore most dyeing processes are restricted in length. Finally, the dyeing temperature was set at 60, 70, 80, 90, and 100°C with fixed dye concentration of 6% w/v and time of 60 min. When the temperature was increased to 80°C , the K/S increased, as shown in Figure 2(c). The temperature had a significant effect on the fabric shade, as thermal energy accelerates dye adsorption and enhances fiber swelling, which in turn increases the amount of dye adsorbed [29–31]. However, when the temperature exceeded 80°C , dye was absorbed more slowly and little change was observed in color intensity. The optimal dyeing condition was found to be 6 g/L of dye extract at 80°C for 60 min.

3.2. Color Measurement. Table 1 shows the L^* , a^* , and b^* values of the fabrics. The untreated hemp fabric had an L^* value of 84.14, compared with 55.42 for the dyed fabric and 51.97 for the antibacterial fabric. This reduction in fabric brightness suggested a greater concentration of dye and antibacterial molecules on the fabric surface. The absorption of dye and antibacterial molecules is also measured by the K/S , and this increased after dyeing. This was confirmed by the SEM images shown as Figure 3. The presence of dye and antibacterial molecules (Figures 3(b) and 3(c)) as a coating on the hemp surface is visible in Figure 3(a). The increase of positive b^* values for dyed and antibacterial fabrics were 30.50 and 30.24, compared with 2.75 for untreated fabric. No significant difference was found in a^* values for all fabrics. These mean that the dyed fabric was yellowish brown in color. The appearance of the all fabrics is shown in Table 1.

FIGURE 2: K/S values against dyeing condition, (a) dye concentration (% w/v), (b) time (min), and (c) temperature ($^{\circ}C$).

TABLE 1: Color values of fabrics after dyeing at temperature $80^{\circ}C$ for 60 min at a dye concentration of 6% w/v and liquor ratio of 1 : 30, and antibacterial finishing condition by 2 dip 2 nip at a pressure of 2.5 kg/cm^2 .

Pretreatment	L^*	a^*	b^*	Color values K/S	Resulting color
Untreated	84.14	2.47	2.75	0.330
Dyed	55.42	3.12	30.50	4.512
Dyed and antibacterial finished	51.97	3.70	30.24	14.077

Figure 4 shows the FTIR spectra of (a) untreated, (b) dyed, and (c) antibacterial finished fabrics. The characteristic peaks of the main components in hemp fabric (cellulose, hemicellulose, and lignin) were observed in all three samples [32]. The broad peaks at 3350 and 2960 cm^{-1} correspond to O-H stretching and C-H symmetrical stretching of cellulose and hemicellulose. The peak at 1745 cm^{-1} corresponds to

the C=O stretching of pectin, waxes, and hemicellulose. The peak at 1690 cm^{-1} corresponds to O-H bending of absorbed water. The peak at 1370 cm^{-1} corresponds to C-H bending of cellulose and hemicellulose. The peaks around $950\text{--}1050\text{ cm}^{-1}$ correspond to the C-C, C-OH, and C-H ring and side groups of cellulose and hemicellulose. As can be seen for Figures 4(b) and 4(c), for the dyed

FIGURE 3: SEM images of (a) untreated, (b) dyed, and (c) antibacterial finished fabrics.

FIGURE 4: FTIR spectra of (a) untreated, (b) dyed, and (c) antibacterial finished fabrics.

and antibacterial finished fabrics, peaks were notably observed at around $550\text{--}600\text{ cm}^{-1}$. These correspond to C-H bending of aromatics [19, 33]. The change of the peak at 3350 cm^{-1} from broad to narrow was attributed to bonding of cellulose with tannin through metallic mordant as proposed in Figure 1 [16]. These suggested satisfactory coverage of the pomegranate extract on the cellulosic structure of the hemp fabric.

3.3. Color Fastness Testing. Pomegranate peel is a rich source of tannins, phenolics, and alkaloids. Tannins are used as coloring compounds and contain both hydrophobic (aromatic) and hydrophilic (-OH) groups, while hemp fabrics also contain hydrophilic (-OH) cellulose groups [34–36]. The mordant, $\text{AlK}(\text{SO}_4)_2$, which is used to fix tannin onto the hemp fabric, does so by forming an insoluble complex. This prevents leaching of dye, producing a stable color after

TABLE 2: Rating of color fastness to washing, water, sea water, acid, and alkaline perspiration of dyed fabrics at temperature 80°C for 60 min at a dye concentration of 6% w/v and liquor ratio of 1 : 30.

Fastness to	Washing	Water	Sea water	Acid Perspiration	Alkaline Perspiration
Color change	3	5	5	5	5
Color staining					
Acetate	5	5	3	5	5
Cotton	5	5	3	4-5	4
Nylon	5	5	3	4	4
Polyester	5	5	3	4	4
Acrylic	3	3	3	3	3
Wool	5	5	4	4-5	4

dyeing. Fabrics with good color fastness do not exhibit color fading under environmental exposure. Color fastness is defined by the degree of color change. Table 2 gives the results of color fastness to washing, water, saline solution (simulated sea water), and perspiration. Color change values were rated as good to excellent (4-5) in all but one test. The exception was the washing test, which was rated as fair (3). However, the color change was not a result of fading, but of a change to brown rather than to yellow. Color stability was also evaluated from color staining, which is defined as excess pickup of dye by a substrate due to exposure to a contaminated medium or by direct contact with a dyed material [28–31]. Table 2 shows the degree of color staining on multi-fiber, cellulose acetate, cotton, nylon, polyester, acrylic, and wool. Each type of fiber contains polar end-groups such as the hydroxyl group (-OH) or amino group (-NH₂), which are able to form H-bond interactions with the phenolic hydroxyl groups of tannin dye molecules that leach out from the samples and contaminate the test medium [36, 37]. All color staining values were rated as good to excellent (4-5), except those of the acrylic fabric which were rated as fair (3). This suggested that formation of the tannin–mordant–acrylic fabric complex was more stable than that of the cellulose-based hemp fabric [34, 35]. Color fastness to crocking was also tested. This involves rubbing the dyed samples. The results are shown in Table 3. The color fastness to crocking of the dyed fabrics was rated as poor (2) in both the dry and wet state, while the fabrics were rated as poor (2) in the dry state and very poor (1) in wet state after washing. As expected, the presence of excess dye molecules on the fabric surface made the fabrics more prone to color loss on rubbing. The use of higher concentrations of mordant may improve dye absorption.

3.4. Antibacterial Activity. Table 4 summarizes the percentage reduction of *S. aureus* (a Gram-positive bacterium) and *K. pneumoniae* (a Gram-negative bacterium) of the dyed, antibacterial finished fabrics before washing and after different washing cycles. Untreated fabric and dyed fabric without antibacterial treatment were used as controls. The pomegranate extract was clearly more active against *S. aureus* than *K. pneumoniae*. The appearance of antibacterial activity is in

TABLE 3: Rating of color fastness to crocking of untreated, dyed and washed fabrics at temperature 80°C for 60 min at a dye concentration of 6% w/v and liquor ratio of 1 : 30.

Properties	Crocking	
	Dry	Wet
Untreated	5	4
Dyed	2	2
Washed	2	1

TABLE 4: Antibacterial activity against *S. aureus* and *K. pneumoniae* of dyed, antibacterial finished fabrics after different wash cycles by percentage reduction (AATCC-100).

Sample	% reduction	
	<i>S. aureus</i>	<i>K. pneumoniae</i>
Untreated	56.67	N.D.
Dyed*	99.67	N.D.
Dyed and antibacterial finished**	99.99	99.99
5 wash cycles	99.99	N.D.
10 wash cycles	99.99	N.D.
15 wash cycles	99.99	N.D.
20 wash cycles	99.99	N.D.

N.D.: no detection. *Dyeing condition: at temperature 80°C for 60 min at a dye concentration of 6% w/v. **Antibacterial finishing condition: padding at a nip pressure of 2.5 kg/cm².

agreement with other results reported for cellulosic fabrics [38, 39]. Pomegranate extract has been claimed to possess strong antibacterial activity because of its chemical composition and biological properties and has been attributed to the presence of tannins and polyphenols. However, it is difficult to attribute antibacterial activity of a complex mixture of bioactive compounds to a single component. It is suggested that the antibacterial effects of pomegranate peel are due to the combined action of several bioactive compounds which can induce bacterial death. Multiple mechanisms acting on specific targets simultaneously have been proposed as an explanation of the inhibitory effect of polyphenolic compounds. These include hydrolysable tannins, which are the main component of pomegranate peel and are associated with the precipitation of bacterial cell membrane proteins by the reaction with protein sulfhydryl groups, making them unavailable for bacterial growth [40, 41].

Durability after repeated washing was evaluated. The effectiveness of antibacterial hemp fabric against *S. aureus* remained at 99.99% across the maximum 20 wash cycles. In contrast, while the effectiveness against *K. pneumoniae* was also 99.99% before washing, the activity disappeared after five wash cycles. These results were confirmed by SEM. As shown in Figure 5, the fabric surface was smooth and clean after washing, suggesting that the loading of antibacterial agents had been reduced. The remaining antibacterial agents were able to resist the growth of *S. aureus*, but not of *K. pneumoniae*. This was attributed to the greater membrane thickness of the Gram-negative bacterium.

FIGURE 5: SEM images of fabrics after (a) 5, (b) 10, (c) 15, and (d) 20 wash cycles.

4. Conclusions

Natural dye was successfully extracted from pomegranate peel using water. The optimal dyeing condition for hemp fabric was found to be 6% *w/v* dye at 80°C for 60 min. This resulted in high color strength values (*K/S*) and a yellowish-brown appearance. In tests of color fastness to washing, water, sea water, and acid and alkaline perspiration, the dyed fabrics were rated as good to excellent. This good resistance to color change is derived from the formation of an insoluble complex of tannin on the fabric surface, which prevents leaching of the dye. Antibacterial agents were successfully extracted using methanol and finished on the dyed fabrics by padding. Strong antimicrobial activity was demonstrated against *S. aureus*, and this was conserved across 20 washing cycles. Although the effectiveness against *K. pneumoniae* was found only before washing, these results clearly demonstrate that the extracts are promising dye materials and that increase antibacterial functionality.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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