The Evaluation of Eucalyptus Leaf Extract for Dyeing and Its Antibacterial Properties on Silk and Wool Fabrics

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Abstract

In an effort to develop antibacterial fabrics, we studied the properties of eucalyptus leaf extract for dyeing and antibacterial activity on natural textiles. Two types of natural fabric, silk and wool, were dyed with 20, 40 and 80% owf of the extract as natural dye using 40% owf of four different types of mordant including Al, Cu, Fe and Sn. The dyed wool and silk fabrics were shown a shade of yellowish-brown except when using Fe mordant which resulted in a shade of dark grayish-brown. Agar diffusion method was performed for inhibition zone observation of dyed fabrics against *Staphylococcus aureus* and *Escherichia coli*, gram positive and gram negative bacteria, as common pathogen. The result showed that the best antibacterial property was observed from wool fabrics dyed with 20% owf dye and 40% owf Cu against both *Staphylococcus aureus* and *Escherichia coli* which were shown 1.26 and 0.98 cm zone of inhibition. Meanwhile, both silk and wool fabrics which were dyed with 40% owf dye and 40% owf Cu, gave the high activity against *Staphylococcus aureus* with 0.88 and 0.86 cm diameter of clear zone. In contrast, these fabrics showed low activity against *Escherichia coli.* This result clearly showed that all dyed fabrics using Cu as mordant showed the antibacterial activity both gram positive and negative strains while other mordants showed less or no activity.

Keywords: Antimicrobial fabric, Eucalyptus extract, Antibacterial activity, Natural fabric Natural dye

1. Introduction

Medical and hygienic textiles in terms of antibacterial fabrics have become important areas in the textile production not only in medical applications but also in daily use. Recently, the antimicrobial agent applied to fabrics have been synthetic or natural substances such as formaldehyde, heavy metal ions (silver, copper), antibiotics and natural dyes [1-3]. There are many reports which showed the antimicrobial activity of natural dyes beside their coloring property including lawsone from henna, juglone from walnut and lapachol from alkanet [4].

Natural dyes have a wide range of shades that can be obtained from insects, minerals, fungi and various parts of plants including root, bark, leaves, flowers, skins, fruits and shells of plants [5-6]. Natural dyes are well known for their uses in coloring of food substrate, leather, wood, as well as natural fibers (wool, silk, cotton and flax) as major area of application since ancient time [7]. Some natural dyes are not only dye with unique and elegant colours but also provide antibacterial, deodorizing and UV protective functions to fabrics [8-14]. Nowadays, the use of natural dyes in textile application has been witnessing a rapid growth. This is mainly attributable to strict environmental standards set by many countries/ organizations to avoid the health hazards associated with synthetic dyes used in textile. The recent ban on the use of azo dyes by European Union has also increased the scope for the use of natural dyes [15].

Eucalyptus is one of the most important source of natural dye that gives yellowish-brown colorants. The coloring substance of eucalyptus has ample natural tannins and polyphenols varying from 10% to 12% [16]. The major coloring component of eucalyptus bark is quercetin as also an antioxidant. It has been used as a food dye with high antioxidant properties [17]. Eucalyptus leaves contain up to 11% of the major component of tannins (gallic and ellagic acids) whil minor substances are flavonoid (quercetin, and rutin, etc.) [18-20]. Tannins and flavonoids are considered very useful substances during the dyeing process because of their abilities to fix dyes within fabrics. Dyeing process using natural dyes, normally requires “mordants”, which are metallic salts of aluminum (Al), iron (Fe), chromium (Cr), copper (Cu), among others, for ensuring a reasonable fastness

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of the color to sunlight and washing [21]. The metal ions of these mordants take action as electron acceptors for electron donors to form coordination bonds with the dye molecules, resulting them insoluble in water [22].

In this study, the dyeing, fastness and antibacterial properties of silk and wool fabrics using an aqueous extract of eucalyptus leaves as natural dye were investigated. Different factors affecting dyeing ability were also thoroughly examined.

2. Meterial and Methods

A. Fabrics

A commercially produced wool fabric (plain-weave) was scoured with an aqueous nonionic surfactant solution at a temperature of 45°C for 30 minutes, then it was thoroughly rinsed, and air dried at room temperature. The scoured and bleached silk fabric (plain-weave) used thoughout this study, was supplied by Chul Thai Silk Co., Thailand.

B. Mordants and Chemicals

The following laboratory-grade mordants were used: aluminium potassium sulfate dodecahydrate ($AlK(SO_4)_2.12H_2O$), ferrous (II) sulfate heptahydrate ($FeSO_4.7H_2O$), copper (II) sulfate pentahydrate ($Cu SO_4.5H_2O$), and stannous chloride pentahydrate ($SnCl_2.5H_2O$). An anionic wetting agent, Altaran S8 (sodium alkylsulfate), and the soaping agent, Syntapon ABA, were supplied by Chemotex Děčin, Czech Republic.

C. Instrument

The mordanting and dyeing were carried out in a dyeing machine (Linitest Type 7421) with programmable time and temperature control. A Datacolor 3890 was employed for the colour strength measurements.

D. Dye extraction from eucalyptus leaves

Fresh eucalyptus leaves (Eucalyptus camaldulensis) were dried in sunlight for one month and crumbled using a blender; and then they were used as the raw material for dye extraction, which was achieved by a reflux technique. Seventy grams of the crumbled eucalyptus leaves was mixed with 1 liter of distilled water and refluxed for 1 hour. The sample was then filtered and the dye solution was separated into two portions, one for evaporating under reduced pressure (rotary evaporator), and another one for dyeing. A crude dye extract of eucalyptus leaves obtained from the rotary evaporator was crumbled with a blender and used for obtaining a standard calibration curve. A dilution of the eucalyptus leaf extract gives a relatively clear solution with a linear dependance on concentration–absorbance relation at an absorption peak (λmax) of 262 nm [23]. The concentration of 20 g/L was calculated from a standard curve of concentrations of the eucalyptus leaf extract dye solutions versus absorbance at the wavelength mentioned.

E. Dyeing method

Pre-mordanting dyeing was used. The dye concentration was varied at 20%, 40% and 80 % on weight of fabric (% owf.) of eucalyptus leaf extract dye, and four types of mordants (Al, Cu, Fe, and Sn) were used with 40% owf. of each concentration of dye. The pH of the dyeing solution was adjusted to 4 with an acetic acid solution. The silk and wool fabrics were dyed at 90°C at a liquor ratio of 1:50 for 60 minutes.

After dyeing, the dyed samples were rinsed with cold water, washed in a bath with a liquor ratio of 1:50 using 1 g/l of the soaping agent, Syntapon ABA, at 80°C for 5 minutes, then rinsed and finally air-dried at room temperature.

F. Evaluation of colour strength and fastness properties

The colour strength (K/S) and CIELAB of the dyed samples were evaluated using a spectrophotometer (Datacolor 3890). All measured samples showed a maximum absorption wavelength (λmax) value at 400 nm. The K/S is a function of colour depth and is calculated by the Kubelka-Munk equation, $K/S = (1-R)^2/2R$, where R is reflectance, K is the sorption coefficient, and S is the scattering coefficient.

G. Bacteria and culture medium

Bacterial strains that were used in this research including *Staphylococcus aureus* and *Escherichia coli*, were supported by Department of Microbiology, Prince of Songkla University, Thailand. The propagation of bacteria and anti-bacterial assay were undergoing using nutrient medium.

H. Propagation of bacterial strains

Nutrient broth and agar were prepared in the usual fashion [24]. Bacterial glycerol stocks of *Staphylococcus aureus* and *Escherichia coli*, were streaked on nutrient agar and incubated at 37°C overnight to get single colony, which will be used for antibacterial test in next procedure.

I. Antibacterial fabrics assay using agar diffusion method

The disk-agar method was performed for antibacterial activity. Natural fabrics, wool and silk, which were use for antibacterial test, were dyed with eucalyptus dye at two different concentration of 20% owf and 40% owf. Each dyed fabric was applied with various mordant types of Al, Cu, Fe and Sn at the 40% owf mordant concentration. The 3-4 bacterial colonies were seeded in 10 ml nutrient broth and incubated at 37°C overnight. The 500 µl of culture was spread over the surface of nutrient agar. Five mm-diameter fabrics were cut and bored in the agar plates. Antibacterial activity was evaluated after incubation at 37°C overnight by measuring diameters of inhibition zone. Original wool and silk were served as negative controls. Each test was performed triplicate for statistic evaluation.

3. Results and Discussion

A. Effect of dyeing conditions

Table 1 shows the colour values of the silk and wool fabrics dyed with eucalyptus leaf extract dye solution. The K/S values increase with an increase of dye concentration. The mordant activity sequence for the silk was $FeSO_4$ > $CuSO_4$ > $AlK(SO_4)_2$ > $SnCl_2$ > without mordanted and $FeSO_4$ > $CuSO_4$ > $SnCl_2$ > $AlK(SO_4)_2$ > without mordanted for the wool fabrics. In all cases, the ferrous sulfate mordant yielded the best dyeing results. The wool fabric dyed with eucalyptus leaf extract showed a higher colour strength than the silk fabric, which is because the wool fabric contains more functional groups than the silk fabric [25].

TABLE I. Colour value of silk and wool fabrics dyed with eucalyptus leaf extract by pre-mordanting and using 40 % owf. of metal mordants at different concentration of the dye

Type of mordant	Dye Conc. (% owf.)	Silk fabric					Wool fabric				
		K/S	L^*	a^*	b^*	[1]Dyed sample	K/S	L^*	a^*	b^*	[1]Dyed sample
Without mordant	20	1.5	75.0	3.4	15.4		4.9	86.4	0.1	14.1	
	40	2.4	70.1	3.9	19.9		8.6	69.1	4.5	23.4	
	80	3.6	66.4	4.8	20.9		11.9	65.2	4.8	25.9	
$AlK(SO_4)_2$ (Al)	20	2.4	79.3	0.7	20.3		6.1	72.7	1.2	9.1	
	40	4.2	75.3	0.8	23.0		9.2	68.1	2.3	29.3	
	80	5.8	71.1	2.1	25.2		12.9	62.4	4.6	30.0	
$CuSO_4$ (Cu)	20	3.6	64.7	5.2	21.8		13.1	47.2	4.6	25.7	
	40	4.1	63.5	5.2	23.0		15.5	45.3	4.3	25.7	
	80	6.2	59.5	6.0	26.3		16.5	43.9	5.1	25.6	
$FeSO_4$ (Fe)	20	6.5	33.7	2.1	-2.7		12.5	35.3	0.6	4.7	
	40	7.4	32.0	2.4	-2.2		16.8	34.3	0.6	5.2	
	80	10.0	28.6	3.3	-0.8		17.2	29.2	0.7	49.0	
$SnCl_2$ (Sn)	20	2.0	83.9	1.9	20.7		10.0	79.4	2.1	49.0	
	40	2.5	81.1	2.9	22.0		12.3	79.3	3.8	49.6	
	80	3.7	78.5	3.4	22.8		15.1	71.9	4.5	42.4	

Note: [1]Dyed sample at 80 % owf.

Ferrous sulfate and copper sulfate mordants are well known for their ability to form coordination complexes and to readily chelate with the dye. As the coordination numbers of ferrous sulfate and copper sulfate are 6 and 4, respectively, some coordination sites remain unoccupied when they interact with the fiber. Functional groups such as amino and carboxylic acid on the fiber can occupy these sites. Thus, the metal can form a ternary complex on which one site with the fiber and the other site is with the dye [26]. Stannous and alum ions form weak coordination complexes with dye; they tend to form quite strong bonds with the dye but not with the fiber, so they block the dye and reduce the dye interaction with the fiber [26].

The obtained value results obtained (Table 1) show that wool and silk fabrics dyed without mordant and with stannous show yellowish-brown and bright yellow colour, respectively. The samples mordanted with alum and copper sulfate produced medium to dark yellowish-brown. With ferrous sulfate, the colour shade was darker and duller. This may be associated with the change of ferrous sulfate into a ferric form by reacting with oxygen in the air. Ferrous and ferric forms coexist on the fiber and their spectra overlap, which results in a shift of λmax and thus consequently a colour change to a darker shade [27]. Additionally, the tannins in the eucalyptus leaf extract combine with ferrous salts to form complexes, which also result in a darker shade of fabric [28].

B. Antibacterial property of dyed fabrics

Natural fabrics including wool and silk were dyed as mention earlier. Its antibacterial activity was studied using agar diffusion method. The result showed that only wool which was dyed with 20% owf and 40% owf of eucalyptus dye concentrations, displayed the antibacterial activity (Table 2) while silk that was dyed at the same condition, had no activity. This result was supported by Bachir and Benali, 2012 [29]. They reported that the essential oil of the eucalyptus leaves has antimicrobia activity against gram-negative bacteria *(E. coli)* as well as gram positive bacteria *(S. aureus)*. However, the structure of fabric can also effect the staining property of dye [30]. The molecular structure of wool has much more amino acids with polar side residues than silk. It affects the dye and heavy metal adsorption capacity of the fabric [31] resulting in improved antimicrobial properties.

TABLE II. THE INHIBITION ZONE OF NATURAL DYE FROM EUCALYPTUS LEAVES COMBINED WITH METAL MORDANT TYPES FOR NATURAL FABRICS STAINED, SILK AND WOOL

Dye	Dye Concentration	Fabric	Mordant	Zone of inhibition (diameter in cm)	
				Staphylococcus aureus	*Escherichia coli*
Eucalyptus Extract	20% owf	Wool	None	0.14	0.20
			Fe 40% owf	0.16	0.22
			Al 40% owf	-	0.34
			Cu 40% owf	1.26	0.98
			Sn 40% owf	0.32	-
		Silk	None	-	-
			Fe 40% owf	-	-
			Al 40% owf	-	-
			Cu 40% owf	0.18	0.68
			Sn 40% owf	-	-
	40% owf	Wool	None	-	0.22
			Fe 40% owf	0.28	-
			Al 40% owf	-	-
			Cu 40% owf	0.88	0.20
			Sn 40% owf	-	0.16
		Silk	None	-	-
			Fe 40% owf	-	0.18
			Al 40% owf	-	-
			Cu 40% owf	0.86	0.48
			Sn 40% owf	-	-

Moreover, the antimicrobial activity of dyed fabrics combined with metal mordants was observed (Table 2). The biggest clear zone was exhibited against both *S. aureus* and *E. coli* in the case of dyed wool with 20% owf of dye and 40% owf Cu mordant. It showed the inhibition zone of 1.26 and 0.98 cm, respectively. Meanwhile, other applied fabrics were presented the antibacterial properties such as wool and silk dyed with 40% owf dye and 40% owf Cu mordant, showed the inhibitory zone of 0.88, 0.86 and 0.20, 0.48 cm against *S. aureus* and *E. coli* (Fig. 1). The suppression activity was also observed from wool dyed with 20% owf dye and 40% owf Fe mordant against *S. aureus* and *E. coli* with the clear zone of 0.16 and 0.22 and slight antimicrobial activity was shown with Al and Sn mordants as well (Table 2). This result reveals that mordant types especially Cu enhanced the antibacterial property of the dyed wool and silk on both positive and negative strains. The explanation likely lies in the increase of charge on the fabric due to the adsorption property of the metal ions (mordant) on the polar amino acids (fabric) [31] cause the interaction between bacterial cell and fabric through Cu [32]. Cu is known as biocide [33] and effective against a wide range of gram-positive and gram-negative bacteria including *Bacillus subtilis, Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* [34-35]. The effect of Cu on *E. coli* cell has also been studied [36]. It describes that Cu ion induces the formation of superoxide anions and hydrogen peroxide and cause DNA damage resulting in cell death (Fig. 2).

4. CONCLUSION

The wool fabric dyed with eucalyptus leaf extract shows higher *K/S* values than the silk fabric. The use of a ferrous sulfate mordant gives rise to the best dyeing and exhibits a darker shade. The silk and wool fabrics dyed with a eucalyptus leaf extract dye solution with or without mordants showed a yellowish-brown shade (except with stannous chloride, which produced the shades of bright yellow). The use of mordants not only improves colour strength, but also provides shade differences.

However, the study of antibacterial activity of these dyed fabrics showed that dye concentration and mordant typed had an effect on antimicrobial property. The combination of dye and mordant typed enhanced the bacterial inhibition of both positive and negative strains. The best condition of antimicrobial fabric was shown on dyed fabric with 40%owf dye and 40%owf Cu mordant giving a 0.8 cm diameter of clear zone. However, other mordants also showed antimicrobial activities: e.g. Fe of 20%owf and 40%owf dye concentration which resisted to *S. aureus* and *E. coli* on wool while Al, Sn and Sn gave a small effect mostly against *E.coli*. Additionally, all dyed wool had better bacterial resistance than dyed silk. This research will be useful for developing antimicrobial textile.

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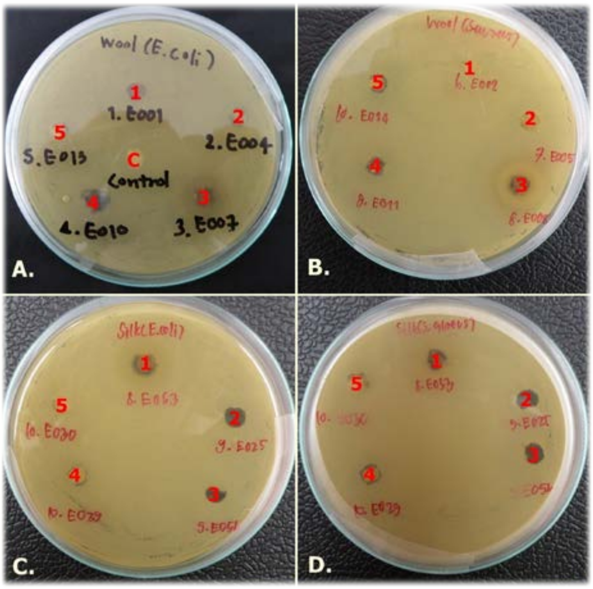

Figure 1 Anti-bacterial activity of wool and silk fabrics with 40% owf dye and 40% owf mordant types including Fe (1), Al (2), Cu (3) and Sn (4) with negative control of no mordant (5). (A) Wool fabrics dyed with 40% dye and 40% owf of mordants on *E.coli* culture and (B) on *S.aureus* culture. (C) Silk fabrics dyed with 40% owf dye and 40% owf mordants on *E.coli* culture and (D) on *S.aureus* culture.

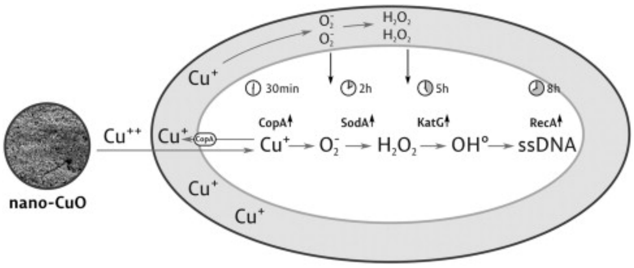

Figure 2 The effect of Cu on E. coli cell [36]

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