SOME APPLICATION OF NATURAL DYE EXTRACTED FROM *EUCALYPTUS GLOBULUS* LABILL (EUCALYPTUS) BARK ON COTTON

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Abstract

The raw sample barks (Eucalyptus) were collected from Aunglan Township, Magway Region. Natural dyes were extracted from the bark of Eucalyptus by water. The prepared natural dye was characterized by FT IR. The phytochemical tests of extracted natural dye were carried out. The maximum wavelength (λ_{max}) of Eucalyptus dyes extracted with water at 483 nm was determined by UV-spectrophotometer. The percentage of bio-mordants was determined. And then, the effect of dyeing time was studied by using colour densitometer at three mordanting methods. The extracted natural dyes were also used on cotton cloth by three mordanting methods. Extracted dye though Specifically identified, from Eucalyptus bark was as adsorbate. Cotton cloth was used as adsorbent. The colour intensities of these dyed cotton cloth was determined by Reflection Transmission Colour Densitometer. Depending on the type of mordant, such as onion peel, jengkol peel and tea waste (bio-mordants), colour fastness of the dyeing cotton cloth were studied. And then, cotton can also be dyed by using post mordanting methods. In addition, the antimicrobial activities of Eucalyptus dye were investigated by Agar Disc Diffusion method on six tested organisms.

Keywords: Natural dye, Eucalyptus bark, mordants, dyeing process

Introduction

The natural dyes have been traditionally extracted from animal and plant sources for use in colouring food substrate, leather, wood and natural fibers such as silk, cotton and flax from time immemorial. The main advantage of using natural dyes is the fact that their source is renewable, biodegradable and reduces environmental impact. They produce very uncommon, soothing and soft shades which are refreshingly different from the strong bright colour produced by synthetic dyes (Chengeto *et al.,* 2016)

Dyes are a kind of magic, a delight to the eye and a joy to use. Even a brief inquiry into the early discoveries and uses of these colouring agents conveys a sense of mystery and glamour. Primitive people in many different parts of the world discovered that certain root, leaf or bark material could be treated to produce colour in a fluid form. Its application was both religious and functional the embellishment of body, clothing and utensils. A dye is a colourant that penetrates the actual fiber and appears to become a part of it. The best colour fastness is attributed to those dyes which must successfully colour the total fiber (Joshi *et al.,* 2015). Most of the natural dyes have no substantively on cellulose or other textile fibers without the use of a mordant. Aqueous extraction of natural dyes was most preferred method by textile dyers. The standardized dyeing techniques are needed for the better commercialization of natural dyes.

Natural dyes have less substantivity to the fiber and have poor fastness properties, hence require a mordant to improve their fixation in the fiber by forming a mordant- dye complex through chemical bonds. Mordants are metal salts which produce affinity between dye and the fiber. Mordants not only help in dye uptake and colour fastness, it also helps in achieving different colour shades in the textiles (Samanta *et al.,* 2009)

The majority of natural dyes need a mordanting chemical (preferably metal salt or suitably coordinating complex forming agents) to create an affinity between the fiber and dye or the pigment molecules of natural colourant. These metallic salts as mordant form metal complexes

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with the fibers and the dyes (Samanta *et al.,* 2011). Bio-mordants are onion peel, jengkol peel and tea waste. Tannin is a widely used as bio-mordant. Tannin was used for dyeing and printing by people from onion peel, jengkol peel and tea waste. Nowadays in most of the countries, natural dyeing is practiced only as a handcraft and synthetic dyes are being used in all commercial dyeing processes. However, with the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes has once again gained interest (Geetha *et al.,* 2013). Natural dyes are known to exhibit better biodegradability, less toxicity, eco-friendly alternative to synthetic dyes and some dyes also medicinal properties.

Materials and Methods

Sample Collection

Eucalyptus bark is the sample used in this study for extraction of dye, which was collected from the Aunglan Township, Magway Region. The part used for the dye extraction was only bark. And then, they were washed with distilled water and dried at room temperature, and made into fine powder. Cotton cloth was purchased from Shwetaung Myoema Market, Bago Region. Bio–mordents (onion, jengkol and tea waste) and cottons were collected from Mindom Myoma Market.

Extraction of Dyes with Water

The barks of Eucalyptus were collected, chopped, dried and ground to fine powder to allow for most intimate contact with solvent. Air dried powder Eucalyptus (10 g) were extracted with each 100 mL of solvent (water) in Sonicator for 3 times each 30 min and filtered. The filtrates were evaporated by distillation semi-dried solid mass at temperature 100˚C. And then, they were dried in oven and were crushed in mortar and pestle for semi-dried solid mass and sieved with 90 μm aperture size. Finally, dye powders of water extract was 38 %.

Determination of Physicochemical Properties and Characterization of Dyes

Physicochemical Properties

Eucalyptus bark powders were washed with water to remove the adhering dirt and impurities. They were dried at room temperature and weighed. The physicochemical characteristics of Eucalyptus bark powder such as moisture content, ash content were determined by AOAC method and pH was determined by pH meter.

Phytochemical Investigation of Eucalyptus Dye

Phytochemical investigation of Eucalyptus dye was performed to determine the presence or absence of phytochemical compounds according to the methods and procedures expressed in the Phytochemical Bulletin of Botanical Society of America (Harborne, 1998).

FT IR Spectroscopy

FT IR measurements were carried out to determine the functional group of natural dye extracted from Eucalyptus. All measurements were carried out in the range of 400-4000 cm^{-1}. The dye samples were measured by using Prekin Elmer GX system, FT IR spectrophotometer.

Determination of Antimicrobial Activities of Natural Dye

The extracted dye solutions were tested with *Aspergillus flavus, Bacillus subtilis, Candida albicans, Pseudomonas fluorescens, Xanthomonas oryzae* and *Echerichia coli* species to investigate the nature of antimicrobial activities by agar disc diffusion Method**.**

Dyeing the Extracted Natural Dye with Cotton Cloth

The pretreated cotton cloth was dyed using extracted dyes (water) from Eucalyptus. The concentration of (1000 ppm) dye solution dyeing on cotton cloth was studied in terms of temperature and pH. The temperature was varied in 40-90 °C and pH was changed in the value of 3-9 by using UV-visible spectrophotometer. And then, the most suitable conditions for dyeing on cotton cloth were selected. The amount of adsorption at equilibrium q_t (mg/g) and Eucalyptus dyes were calculated by this equation:

$$q_t\,(\text{mg/g}) = \frac{\text{Co}-\text{Ce (mg/L)}}{\text{unit mass of } adsorbent\ (g)} \times \text{volume of solution (L)}$$

where, q_t = adsorption capacity (mg/g), C_o = initial concentration (mg/L)

C_e = equilibrium concentration (mg/L), unit mass of adsorbent = 1 g

Effect of Temperature

Bath adsorption experiments were conducted by 1 g of cotton cloth to 100 mL of dye solutions with water in a 250 mL beaker with a temperature control of 80 °C ± 5 °C. The original pH was used. A 100 mL dye solution in a 250 mL beaker was put in water bath. Natural dye solution dyeing on cotton cloth was allowed to reach the equilibrium for 60 min in a water bath at 40, 50, 60, 70, 80 and 90 °C. At 10 min intervals, the dye solution was taken out from the beaker. The remaining dye concentration was determined by UV-visible spectrophotometer at λ_{max} 483 nm for watery extracted dye. The results were shown in Figure 3.

Effect of pH

The effect of pH on dyeing the cotton cloth with the extracted dye was conducted by the same procedure for equilibrium over a range of pH values (3, 4, 5, 6, 7, 8 and 9) which were adjusted with HCl and NaOH. The results were illustrated in Figure 4.

Extraction of Bio-mordants

The raw onion, tea waste and jengkol peel (10 g) each was extracted with distilled water (1 L) and then setup was kept for 60 min boiling. The extracted tannin was filtered and was used for mordanting.

Pretreatment of Cotton Cloth

The cotton cloth was soaked in mixture of 1g / L of sodium carbonate and 2.5 g / L of detergent at 80 °C for 30 min and then washed with running tap water to remove the natural impurities and improve the texture of cotton cloth for dyeing.

Determination of Dyeing Time on Cotton Cloth by Three Mordanting Methods

The dyeing time were studied by using three mordanting methods (pre-mordanting, simultaneous mordanting and post mordanting). The dyeing time were determined by using colour densitometer. The suitable dyeing time were selected for dyeing methods.

Dyeing Procedure for Cotton Cloth

The pretreated cotton cloth was dyed using water extracted dye from Eucalyptus bark and selected 20 % of bio-mordant (onion peel, jengkol peel and tea waste) at the optimum temperature 80 °C, dyeing time 60 min and pH 6 by using pre-mordanting, simultaneous mordanting and post-mordanting methods.

Determination of Colour Density for Dyeing Cotton on Colour Fastness

The colour density of dyeing cotton cloth before and after sun exposure and washing were determined by Reflection Transmission Colour Densitometer at Universities' Research Centre, Yangon (cf. section 3.6)

Some Application of Natural Dye Extracted from Eucalyptus Bark Dyeing on Yarn

The cotton was collected from Mindom Myoma Market, Magway Region. Firstly, the cotton was soaked with water to remove the dirty and impurity at overnight. The cotton (2 g) was dyed with 20 % onion peel, jengkol peel and tea waste (bio-mordants) and 3 g w/v of extracted dye powder at 80 °C for 1 hr by firing. And then, the cotton was dried in sunlight at 4 hr and the dried yarn was trampled with rice to stiff for weave. The next is firmed step. Finally, the firm fibers were obtained. The weaving steps were shown in Figure 9.

Results and Discussion

The physicochemical characteristics of Eucalyptus bark powder and phytochemical investigation of water extracted dye sample were determined. Table 1 indicates that the results of 7.45% w/w moisture content, 3.25 % w/w ash content and pH 6.50 in raw sample were observed.

Table 1 Physicochemical Properties of Eucalyptus Bark Powder

No.	Characteristic	Content
1	Moisture Content (%)	7.45
2	Ash Content (%)	3.25
3	pH	6.50

Phytochemical Constituents of Eucalyptus Dye

According to the results of phytochemical analysis, it was found that alkaloids, flavonoids, glycosides, phenolic compounds, tannins, α-amino acids and saponins were present in the extracted Eucalyptus dye sample whereas carbohydrates, steroids and starch were absent in this extracted dye sample.

FT IR Analysis

Figure 1 show the FT IR spectrum of natural dyes extracted from Eucalyptus with water. The characteristic absorption bands at 3300, 2885, 1616, 1444 and 1049 cm^{-1} were observed. These peaks correspond to the groups present in the sample and are indicated to O-H stretching, C-H stretching, C=O stretching, C-H bending and C-O stretching which is the good correlation with that of literature. These bands were confirmed the presence of alkaloids, tannins and flavonoids in natural dye (Silverstein *et al.*, 2003).

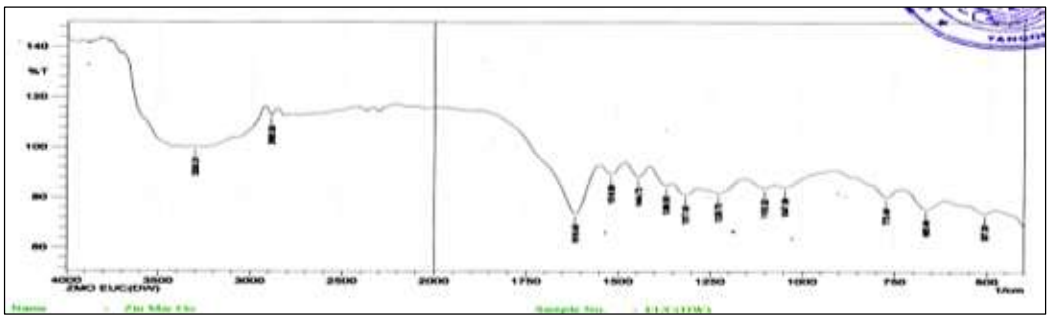
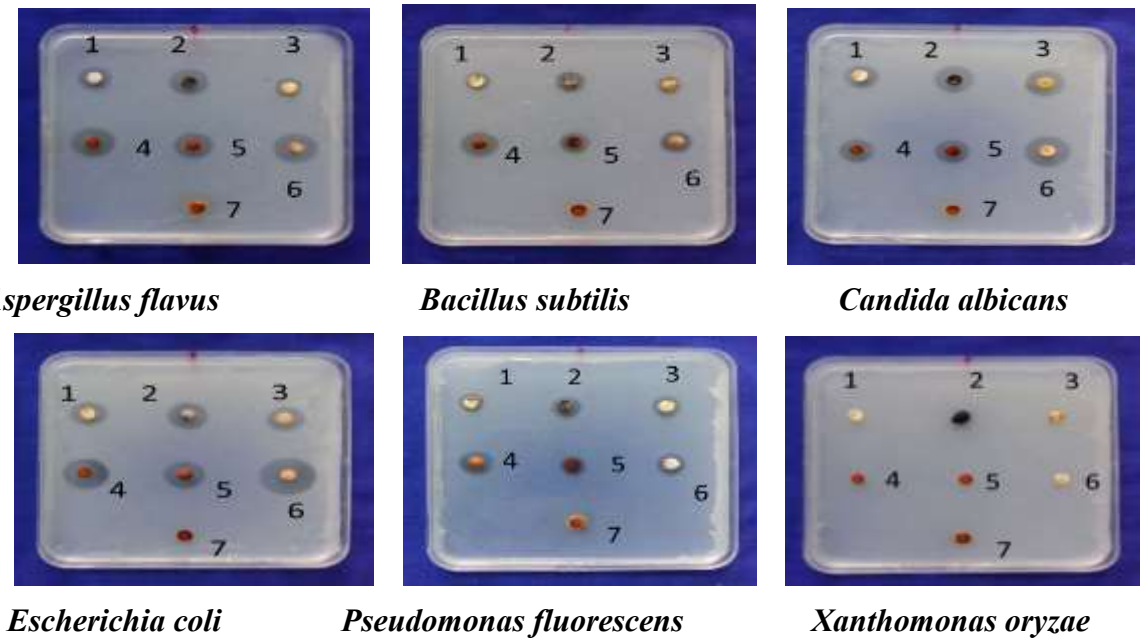


Figure 1 FT IR spectrum of natural dyes extracted from Eucalyptus with water

Antimicrobial Activities of the Extracted Dye from Eucalyptus

It was important to study the antimicrobial activity on dyes extracted from Eucalyptus because natural dyes showed inhibition effect against test bacterial in solution. The results were shown in Table 2 and Figure 2. Among these solvents, watery extract did not show antimicrobial activity against test organisms. Petroleum ether extract showed the highest activity while acetone, ethyl acetate and methanol extract exhibited the lowest activity against six types of microorganisms. The antimicrobial activity might be due to ellagic acid and tannin components.



Aspergillus flavus ***Bacillus subtilis*** ***Candida albicans***

Escherichia coli ***Pseudomonas fluorescens*** ***Xanthomonas oryzae***

Figure 2 `Antimicrobial activities of various solvent extracts of Eucalyptus dye (1.acetone 2.chloroform, 3.ethylacetate, 4.ethnaol, 5.methanol, 6.pet.ether and 7. water)

Table 2 Antimicrobial Activities of Eucalyptus Dye

No.	Test Organisms	Acetone	$CHCl_3$	EtOAc	EtOH	MeOH	Pet.eth	H_2O
1	*Aspergilus flavous*	+(10)	+(12)	+(8)	+(14)	+(12)	+(14)	-
2	*Bacillus subtilis*	+(8)	+(10)	+(8)	+(14)	+(10)	+(12)	-
3	*Candida albicans*	+(12)	+(14)	+(14)	+(12)	+(12)	++(16)	-
4	*Escherichia coli*	+(14)	++(16)	+(14)	++(18)	++(16)	+++(22)	-
5	*Pseudomonas fluorescens*	+(8)	+(10)	+(10)	+(12)	+(8)	+(10)	-
6	*Xanthomonas oryzae*	-	-	-	-	-	-	-

Agar Disc Diffusion Method -6 mm, 6 mm ~ 12mm (+), 15 mm ~ 19mm (++), 20 mm above (+++), No activity (-)

Sorption of the Extracted Natural Dye from Eucalyptus on Cotton Cloth

Effect of temperature

The adsorption properties of water extracted dye were studied at different temperatures (40-90 °C). In dyeing, the optimum temperature of extracted natural dyes on cotton cloth was 80 °C.

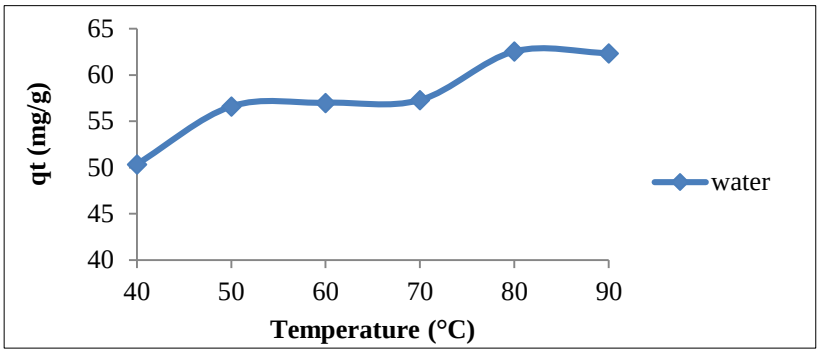


Figure 3 Effect of temperature on dyeing of Eucalyptus dyes extracted by water

Effect of pH

The original pH of extracted dyes (water) was 6.50. The pH values of extracted natural dye were adjusted with 1 % HCl and 1 % NaOH to reach the pH values of 3, 4, 5, 6, 7, 8 and 9. The optimum pH of extracted natural dyes was 6.

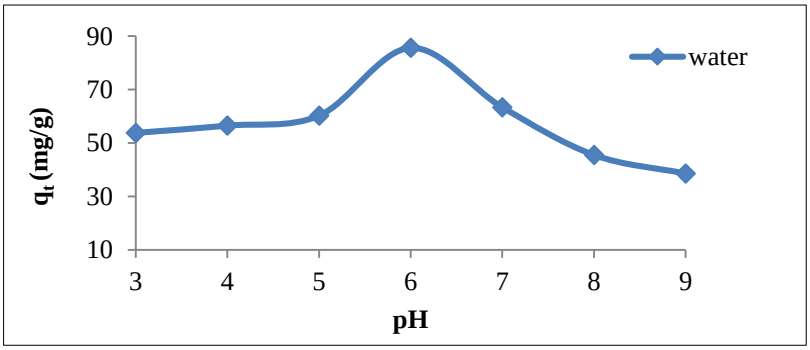


Figure 4 Effect of pH on dyeing of Eucalyptus dyes extracted by water

Determination of Dyeing Time by Three Mordanting Methods

Table 3 shows the suitable percent of bio-mordants. According to the Tables (4, 5, 6 and 7), the effect of dyeing time were studied by using three mordanting methods (pre-mordanting, simultaneous mordanting and post mordanting). The suitable amount of dyeing time was observed at 60 min for three mordanting methods.

Table 3 Colour Density of Extracted Natural Dye with Different Bio-mordants

No.	Volume of Natural dye (mL)	Percentage of Bio-mordants (%) v/v	Colour density/ mordant		
			Onion peel	Jengkol peel	Tea waste
1	100	10	0.35	0.38	0.36
2	100	**20**	**0.76**	**0.74**	**0.70**
3	100	30	0.41	0.44	0.42

- Suitable percent of bio-mordants, size of cotton=3.5”length x 3.5’’ wide,

  Dyeing temperature= 80 °C

Table 4 Effect of Dyeing Time on the Amount of Extracted Eucalyptus Dye on Cotton Cloth Using Direct Dyeing Method

No.	Volume of Natural Dye (mL)	Dyeing Temp (°C)	Dyeing Time (min)	Colour densities
1	100	80	40	0.32
2	100	80	50	0.33
3	100	80	**60**	**0.36**
4	100	80	70	0.34

- Suitable dyeing time, Amount of bio-mordants =20 %, size of cotton =3.5" length x 3.5' wide

Table 5 Effect of Dyeing Time on the Amount of Extracted Eucalyptus Dye on Cotton Cloth Using Pre-mordanting Method

No.	Volume of Natural Dye (mL)	Dyeing		Colour Densities		
		Temp (°C)	Time (min)	Onion peel S_2	Jengkol peel S_3	Tea waste S_4
1	100	80	40	0.36	0.32	0.34
2	100	80	50	0.38	0.35	0.35
3	100	80	60	**0.47**	**0.42**	**0.38**
4	100	80	70	0.42	0.39	0.36

- Suitable dyeing time, Amount of bio-mordants =20 %, size of cotton =3.5" length x 3.5' wide

Table 6 Effect of Dyeing Time on the Amount of Extracted Eucalyptus Dye on Cotton Cloth Using Simultaneous Mordanting Method

No.	Volume of Natural Dye (mL)	Dyeing		Colour Densities		
		Temp (°C)	Time (min)	Onion peel S_2	Jengkol peel S_3	Tea waste S_4
1	100	80	40	0.46	0.40	0.36
2	100	80	50	0.48	0.42	0.38
3	100	80	**60**	**0.50**	**0.43**	**0.41**
4	100	80	70	0.47	0.37	0.39

- Suitable dyeing time, Amount of bio-mordants =20 %, size of cotton =3.5" length x 3.5' wide

Table 7 Effect of Dyeing Time on the Amount of Extracted Eucalyptus Dye on Cotton Cloth Using Post Mordanting Method

No.	Volume of Natural Dye (mL)	Dyeing		Colour Densities		
		Temp (°C)	Time (min)	Onion peel S_2	Jengkol peel S_3	Tea waste S_4
1	100	80	40	0.45	0.42	0.37
2	100	80	50	0.47	0.43	0.38
3	100	80	**60**	**0.53**	**0.45**	**0.42**
4	100	80	70	0.50	0.41	0.40

- Suitable dyeing time, Amount of bio-mordants =20 %, size of cotton =3.5" length x 3.5' wide

Colour Fastness Properties of Eucalyptus Dye Extracted with Water Dyeing on Cotton Cloth

Colour density on the cotton cloth was increased significantly when a mordant was used. The size of cotton was used 3.5" length x 3.5' wide for dyeing process. The colour fastness cotton cloth samples were prepared using pre-mordanting, simultaneous mordanting and post mordanting, 20 % v/v dye concentration, pH 6, temperature at 80 °C and 60 min dyeing time because those conditions resulted in the highest colour strength for cotton cloth. The suitable amount of bio-mordants was selected by colour densities. The effect of dyeing time on the amount of extracted Eucalyptus dye on cotton cloth were studied by using direct dyeing method, pre-mordanting, simultaneous and post mordanting method. Among three mordanting methods, post-mordanting method was the best for dyeing process. In this, the maximum dyeing time were also studied by colour densities. And then, using the above optimum conditions, the colour density for Eucalyptus dye extracted with water solution dyeing on cotton cloth before and after colour fastness testing were compared in Figures 5, 6,7 and 8. For the dyeing on cotton cloth, the dyed cotton cloth without mordant was seen the lowest colour density. Among mordants, natural mordant (onion peel) was the highest colour density whereas jengkol and tea waste were nearly equal colour density. It was also found that onion peel was good in colour fastness (Li *et al*., 2016).

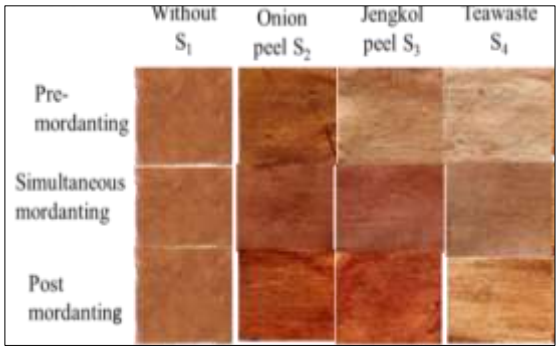


Figure 5 Colour of cotton cloth dyeing water extract of Eucalyptus dye (i)pre-mordanting, (ii)simultaneous mordanting and(iii) post mordanting

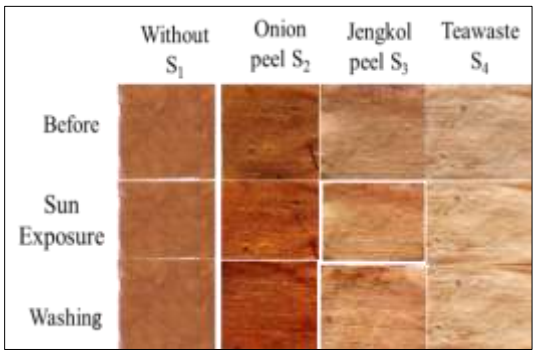


Figure 6 Colour of cotton cloth dyeing water extract of Eucalyptus dye by pre- mordanting method

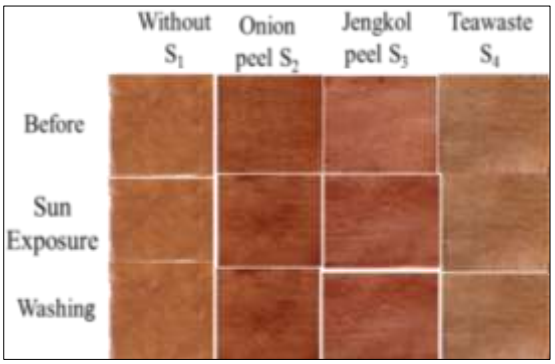


Figure 7 Colour of cotton cloth dyeing water extract of Eucalyptus dye by simultaneous mordanting method

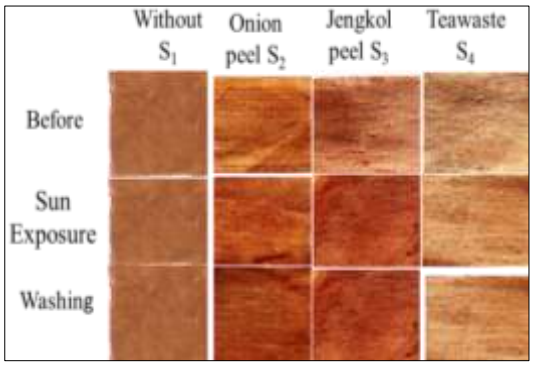
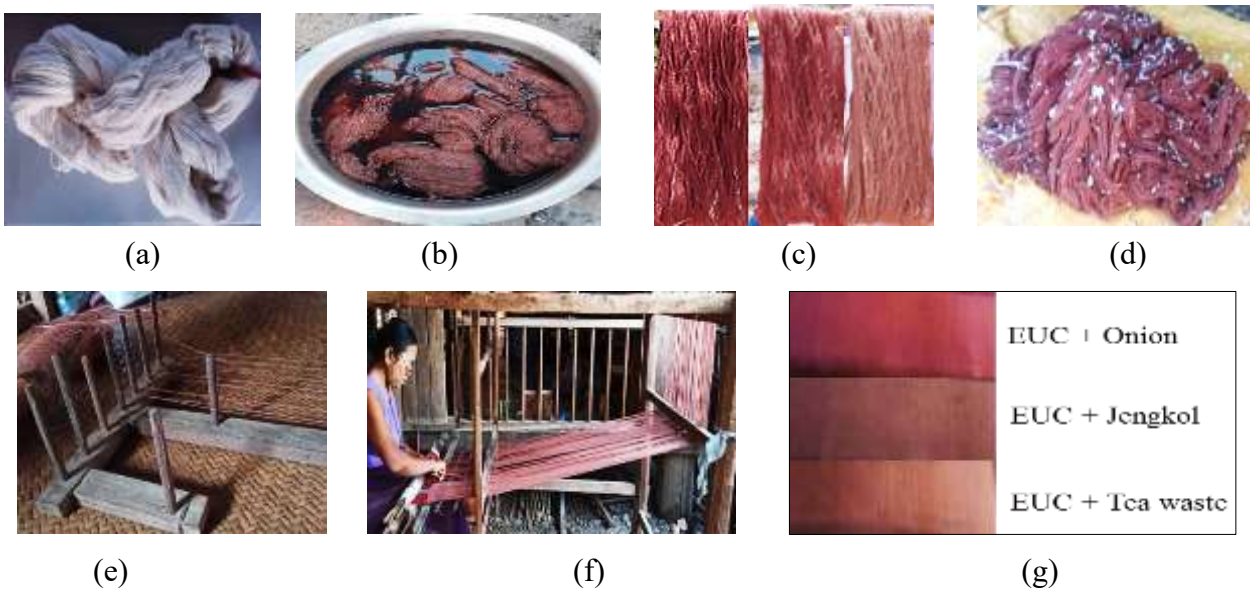


Figure 8 Colour of cotton cloth dyeing water extract of Eucalyptus dye by post mordanting method

Some Application of Natural Dye Extracted from Eucalyptus using Bio-mordants on Cotton

Cotton was dyed, using three types of bio-mordants with dyes obtained from Eucalyptus and colour fastness was compared. They were applied to dye not only cotton cloth but also pure cotton. Post mordanting method was used that gives the outcome of the best colour fastness and the dyed cotton can be seen in Figure (c). After that, stiffen the cotton using rice for weaving is as shown in Figure (d). In Figures (e) and (f), the cotton for weaving is knotted to a tie. The cotton weaved by using three types of bio-mordants are shown in Figure (g). The two courses of dyeing process show that weaving cotton cloth with purely dyed cotton is lighter in tone than to dye cotton cloth after weaving.



(a) (b) (c) (d)

(e) (f) (g)

Figure 9 Weaving steps of the cotton by the extracted natural dye from Eucalyptus with water (a) cotton (b) dyeing, (c) drying, (d) trample, (e) tiding, (f) weaving and (g) cloth

Conclusion

Natural dyes were extracted from Eucalyptus bark powder with water. The physicochemical properties of Eucalyptus raw bark were investigated. In phytochemical test, tannins, steroids, alkaloids, flavonoids, glycosides, phenolic compounds, saponins and α-amino acids were observed in the extracted dyes of water whereas carbohydrates and starch were absent in this extracted dye. According to the FT IR analysis, Eucalyptus dyes extracted by water, functional groups of O-H, C-H, C=O (stretching), - CH bending and C-O stretching were observed. . Furthermore, antimicrobial activities of acetone, chloroform, ethyl acetate, ethanol, methanol, petroleum ether and water extracted dye from Eucalyptus were investigated by agar disc diffusion method. Among these extracted dyes, watery extract did not show antimicrobial activity in all test organisms. If the dyed cotton wears, there were not be toxic on human skin and other effectives. Thus antimicrobial activities were also tested. The maximum absorption wavelength (λ_{max}) of Eucalyptus dyes extracted by water was 483 nm. The suitable amounts of bio-mordants were selected by colour densities. The effect of dyeing time on the amount of extracted Eucalyptus dye on cotton cloth were studied by using direct dyeing method, pre-mordanting, simultaneous and post mordanting method. The colour fastness of dyed samples was determined by three mordanting methods. Among three mordanting methods, post-mordanting method was the best for dyeing process. For the dyeing on cotton, the dyed cotton without mordant was seen the lowest colour density. Among mordants, bio-mordants (onion) were highest colour density whereas jengkol and tea waste were nearly equal colour density. Further, cotton can be dyed by traditional methods. The cotton is

steadier colour than cotton cloth. Thus, not only the cotton cloth but also cotton can be dyed. Nowadays most of the natural dyers are interested to use the natural dye materials in the same ways used for synthetic dyes.

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