Extraction and Utilization of Natural Dye from the Bark of Babul (*Vachellia nilotica*) as Antimicrobial Finish for Cotton Fabrics

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Abstract

In this research, the Babul (Sha) bark from the campus of Myingyan University, Myingyan Township, Mandalay Division was used for the preparation of natural dyes. In this study, the phytochemical investigations of the bark were done by the test tube method. Moreover, the antimicrobial activities of the ethanol and water extract of the bark were performed by agar well diffusion method on three tested microorganisms. The functional groups present in the extracted dye sample were characterized by FT IR spectrophotometric analysis. Furthermore, the dyeing methods such as pre-mordanting, simultaneous mordanting, and post-mordanting were used in dyeing cotton. As mordant, Potash Alum [$K_2Al_2(SO_4)_3.24H_2O$], Ferrous Sulphate ($FeSO_4$), and Copper Sulphate ($CuSO_4$) were used. The mordanting procedures were followed the same for all the experiments. Finally, the color fastness like color fastness to washing, colorfastness to perspiration/saliva, colorfastness to water, colorfastness to rubbing, and colorfastness to light were checked and found satisfactory results. Staining and changing in color were assessed by using a standard greyscale and the results were recorded. The overall results showed that an extracted dye from Babul (Sha) bark was suitable for reducing harmful and toxic to the environment and also for humans.

Keywords: Natural dyes, mordant, cotton fabric, fastness test

Introduction

Natural dyes are obtained from renewable resources of nature, such as plants and animals, although natural dyes from minerals of the earth are also known. Coloring matters obtained from various inorganic metal ores and metal salts are called mineral dyes. Natural dyes find application chiefly for the coloration of food, drugs, cosmetics, and textile. Some quantities of dyes are also used for the coloration of paper, leather, shoe polish, candle, wood, etc. The use of natural dyes for the coloration of textiles is practiced since the early days. Nature provides rainbow colors that show up in everyday life. The art of making dyes from plants dates back centuries those colors and the plants they are made from survive till now. These metallic mordants after combining with dye in the fiber, form an insoluble precipitate or flake and thus both the dye and mordant get fixed to become wash fast to a reasonable level (Ghorpade *et al.,* 2000, Kumeresan, *et al.,* 2011). The word 'natural dye' covers all the dyes or colorants derived from natural sources like different parts of various tree species and plants, invertebrates, and minerals (Konar *et al*., 2011). Scientifically, dyes are defined as chemical compounds that are attracted to substrates in a more or less permanent state, and evoke the visual sensation of color. They can further be defined as molecules that have the ability to absorb and reflect the visible part of light at a specific electromagnetic spectrum to give the human eye a sense of color (Institute of Chemical Technology). Such dyes that are majorly from plant parts are extracted by either the aqueous method, acids, or basic solvent method (Antima *et al*., 2012; Ponmozhi *et al*., 2011). The dye extracted from these sources usually has low to medium fastness; therefore, improvement in their shade can be archived through the use of 12 mordants (Konar *et al*., 2011, Saxena *et al*., 2014).

There are diverse types of mordants that have been applied on textile substrates which on application give different results with the same dye or different

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classifications of dyes. Natural dyes have been classified in several ways depending on their unique chemical structure and particular way of bonding that is, chemically where the substrate bonds strongly with the dye compounds or are held by physical forces. Unlike synthetic dyes with a single entity, the structure of natural dyes comprises complex chemical constitutions with mixtures of closely related chemical compounds.

Botanical Description of Babul (Sha)

Common name	-Babul
Botanical name	- *Vachellia nilotica*
Family	- Fabaceae
Genus	- *Vachellia*
Kingdom	-Plantae
Part used	-Bark

Materials and Method

Sample Collection and Preparation

The Babul (Sha) bark was collected from Myingyan University Campus, Myingyan Township, Mandalay Division, Upper Myanmar. It was washed with distilled water and cut into small pieces and dried in air for two weeks. They were made powdered by a grinding machine and then stored at room temperature for further use.

Preliminary Phytochemical Test on Babul (Sha) Bark

Before the extraction of natural dyestuff, the preliminary phytochemical examination was carried out on a dried powdered sample to determine the presence or absence of alkaloids, flavonoids, reduction sugars, terpenes, glycosides, steroids, phenolic compounds, tannins, and saponins (Tin Wa, 1972).

Screening of Antimicrobial Activity of the Crude Extracts of Sha Bark by Paper Disc Diffusion Method

The antimicrobial activity of the crude extract such as ethanol, and water of Babul bark was determined against three strains of microorganisms such as *Candida albicans, Pseudomonas fluorescens, and Staphylococcus aureus* by employing the agar well diffusion method. The tests were screened at the Department of Chemistry, Meiktila University

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FT IR spectrophotometric analysis of the Babul bark

FT IR spectrophotometric analysis of Babul bark was done at the Department of Chemistry at Meiktila University by using the Thermo Scientific Nicolet Summit FT IR Spectrometer system, and FT IR spectrophotometer was used to identify the functional groups present in the dye sample (Silverstein. R.M. *et al.*, 1998). The result was described in Figure 3.

Extraction of Natural Dye from the barks

The barks were used in this study for the extraction of dye. About 25 g of the barks with 400 mL distilled water and boiled at 60ºC for 30 minutes to obtain the dye solution of 200 mL. This dye solution was cooled and then filtered with a filter cloth. It was used for the dyeing of cotton fabrics.

Dyeing Process on Cotton Fabrics

(1)Mercerization of Cotton Fabrics

Mercerization was done according to the standard procedures (Konar *et al*., 2011). Firstly, 5 g of sodium hydroxide was dissolved in 2 L of warm water before adding to the cotton fabrics. To prepare mercerized cotton fabrics, 1 yard of cotton fabric (about 80 g) was simmered in sodium hydroxide solution for 60 min and rinsed with water. After completion of this step, the cotton fabric was put into a vinegar solution (4mL acetic acid and 1L of water) for 30 min to neutralize it. To prepare clean cotton fabrics, the cotton fabrics were rinsed with water and then dried at room temperature. Further, it was cut into many cleaned fabrics with about 1.7 g.

(i) Pre-Mordanting Method

In pre-mordanting, firstly the scoured and bleached fabrics were treated with suitable mordant. The mordant solution was prepared by heating 1.25 g of alum with 200 mL of water to obtain a volume of material to liquor ratio of 10:200. This solution was heated for 15 minutes at 80º C and 10 g of cleaned fabrics were immersed in the above mordant solution and continued to heat. During mordanting, these fabrics were frequently stirred to obtain good penetration of mordants into them. After that, these fabrics were dried. After mordanting, these mordanted fabrics were immersed in a dye bath containing water extract of Babul bark for about 30 minutes. One of three fabrics was originally allowed to keep. One was rinsed with water and one was rubbed. The same experiment as the alum was also carried out with another mordant $CuSO_4$ and $FeSO_4$. The comparison of the color results was shown in Figure 4.

(ii)Simultaneous- Mordanting Method

200 mL of concentrated dye liquid was added to a beaker containing 1.25 g alum to make a volume of material to liquor ratio of 10:200. Then the dye bath was heated at 80º C and 10 g cleaned fabric was simmered in that solution for about 30 minutes. After that, the fabric was rinsed with water and allowed to air dry. The sample experiment was carried out with different mordants such as $CuSO_4$ and $FeSO_4$.

(iii)Post-Mordanting Method

With regard to the post-mordanting method of natural dyeing, the dyeing process was carried out on the last three cleaned fabrics in the dye bath without mordant. 200 mL of prepared concentrated dye liquid was heated at 80º C and 10 g of cleaned fabric was simmered in this solution for 30 minutes. The fabric was frequently stirred with a glass rod to obtain good penetration of dye molecules into the fabric. The cotton fabric was rinsed with water and subjected to air drying. After dyeing, the mordanting bath was prepared by heating 1.25 g alum with 200 mL water to obtain material to liquor ratio of 10:200. This solution was heated to 80º C and 10 g of cleaned fabric was simmered in it for about 45 minutes. After that, similar experiments were carried out as a pre-mordanting method. Different mordants such as $CuSO_4$ and $FeSO_4$ were used for the dyeing process.

Color Fastness of Dyed cotton with the sample by Launder Washing and Rubbing the Tester

(1) Determination of color fastness on dyed cotton

After dyeing the cotton with and without mordants, color fastness tests are carried out to determine the fastness properties of the dye sample. In this study, the dye sample was rated in terms of the color fastness to washing and rubbing. Test for rubbing fastness was carried out in dry and wet conditions and the washing fastness of the dye sample was determined by studying the change of shade and staining on

cotton. These measurements were done at the development center of Textile Technology in the Ministry of Industry, Yangon Region, according to International Standard Organization (ISO) Test no.2.

(2) Determination of fastness to washing on dyed cotton

In this experiment, the Launder Tester washing machine (Model number L.7501) is used to determine the fastness in the washing properties of dyed cotton. This operation is carried out under ISO Test 2 method. The test specimen was cut into 10cm × 10cm from the dyed cotton and placed between the two pieces of undyed white samples. These three pieces are held together by stitching the edges. The required soap solution was prepared with 5 grams of soap per liter. Then, the soap solution was added to the test bottle. After that, the sandwich fabric was placed in the test bottle and treated at 50 ℃ for 45 minutes. After finishing the washing operation, the test specimen was rinsed in cooled water and dried at room temperature. The Launder Tester washing machine (Model number L.7501) was the change in color of the dyed specimen and staining of adjacent fabric are assessed with greyscales and the results of grading were shown in Table 3.

(3) Determination of fastness to rubbing on dyed cotton

In determining the rubbing fastness of the dyed cotton, the GAKUSHIN type rubbing fastness tester was used. Rubbing tests were performed in the dye and wet state. In the dry state of the rubbing fastness test, the test specimens were cut into 2.5cm × 25cm and mounted onto the curved surface of the machine. The white cotton to be rubbed was cut into a measurement of 7cm × 7cm. And then they were mounted over the end of the finger and the covered fingers were lowered onto the test specimen. The test specimens of white cotton were rubbed together on the tester with the aid of a motor. Rubbing was done 100 times with a 500g load. The wet state of the rubbing fastness test, the procedure of the rubbing fastness test in the wet state was the same as in the dry rubbing fastness test except the cotton to be rubbed is wetted with water. When rubbing was completed, the fabric was dried at room temperature. Finally, the staining of the white cotton sample was assessed by comparing it with the greyscale and the results were summarized in Table 3 (Clark, 2011).

Table 1. Description of Color Fastness to Washing and Rubbing

Fastness Grade	Shade Change of Tested Sample	Terms of most other properties	Staining of Adjacent White Sample
5	No Change	Excellent	No Staining
4	Slightly Changed	Good	Very slight staining
3	Noticeable Changed	Fair	Moderate staining
2	Considerably	Poor	Significant staining
1	Much Changed	Very Poor	Deep staining

Results and Discussion

Preliminary Phytochemical Investigation of the Babul (Sha) Bark

A preliminary phytochemical analysis was performed in order to know the different types of chemical constituents present in the selected dye sample. It was found that, flavonoids, terpenes, steroids, phenolic compounds, tannins, and saponins were present. And then, alkaloids were present by using Wagner's reagent. But reducing sugars, and glycosides were absent in Babul (Sha) bark.

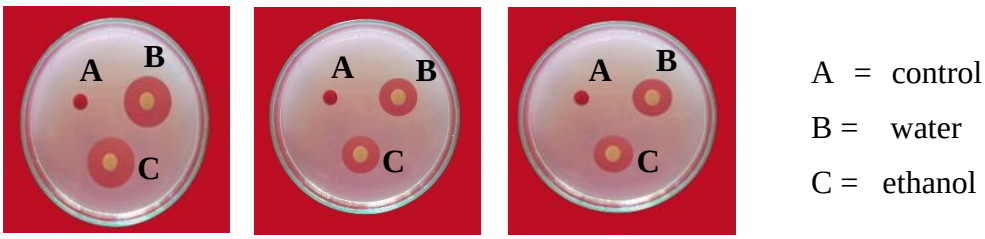
Screening of Antimicrobial Activity of Crude Extracts by Agar Well Diffusion Method

Table 2. Antimicrobial Activities of Babul

Microorganisms	Babul		Control
	H_2O extract	EtOH extract	
Candida albicans	+++	+++	-
Pseudomonas fluorescens	+++	+++	-
Staphylococcus aureus	+++	+++	-

8mm-12mm(+) 13mm – 17 mm (+ +) 18mm – above (+ + +) (-) = absence

It was found that all extracts of bark showed antimicrobial activities on all tested microorganisms. The water and ethanol extracts of the sample exhibited the highest activity against all tested microorganisms. The activity of fabric to resist disease transmission is considered to be an important inevitable parameter for garments that come in direct contact with the human body.



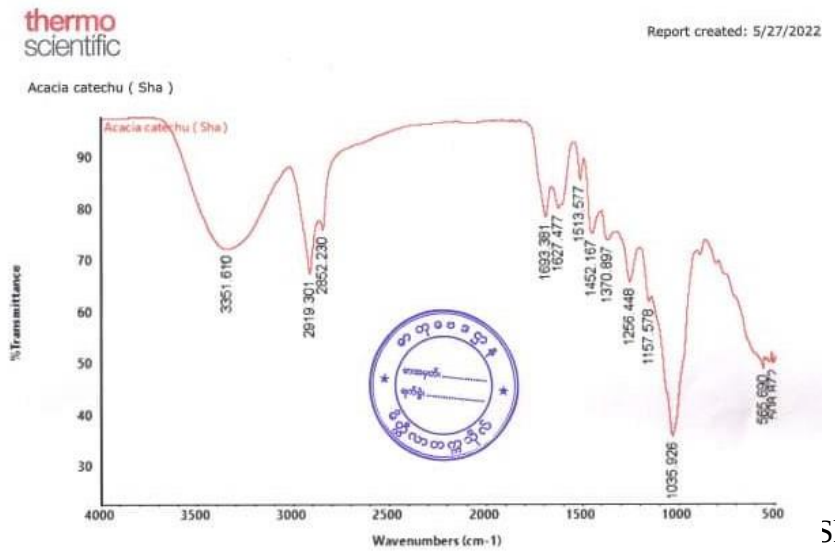
Candida albicans *Pseudomonas fluorescens* *Staphylococcus aureus*

Figure.2 Antimicrobial activities of water and ethanol extract of Babul (Sha)

Characterization of FT IR spectrum of extracted dye sample from Babul (Sha) bark

In the FT-IR spectrum of *Vachellia nilotica* from the bark sample, the band (3351.610 cm^{-1}) was due to the O-H stretching vibration of alcohol groups. The band (2919.301, 2852.230 cm^{-1}) was the C-H stretching vibration of sp^3 hydrocarbon. The two intent bands (1693.381, 1627.477 cm^{-1}) were the C=O stretching vibration of the carbonyl (>C=O) group and the C=C ring skeletal stretching vibration of an aromatic ring. The band (1513.577 cm^{-1}) was the C=C stretching vibration of the alkenes group. The bands (1452.167, 1370.897 cm^{-1}) were the C-H in-plane bending vibration of the alkenes group. The band (1256.448 cm^{-1}) was the C-H in-plane bending vibration of the gem dimethyl group. This spectrum also represented the C-O stretching vibrating of the alcohol group at (1157.578 cm^{-1}) and, (1035.926 cm^{-1}) at C-O-C stretching vibration of the ether functional group. Moreover, the mono-

substituted phenyl groups could be detected at (565.690, 508.872 cm^{-1}). According to the FT IR spectrum, the extracted dye contains OH group, sp^3 hydrocarbon, aromatic benzene ring, C=O group and C-O-C group. These bands confirmed the presence of alkaloids, tannins and flavonoids in natural dye.



Sha) bark of (i) Pre-mordanting (ii)Simultaneous-mordanting (iii) Post-mordanting

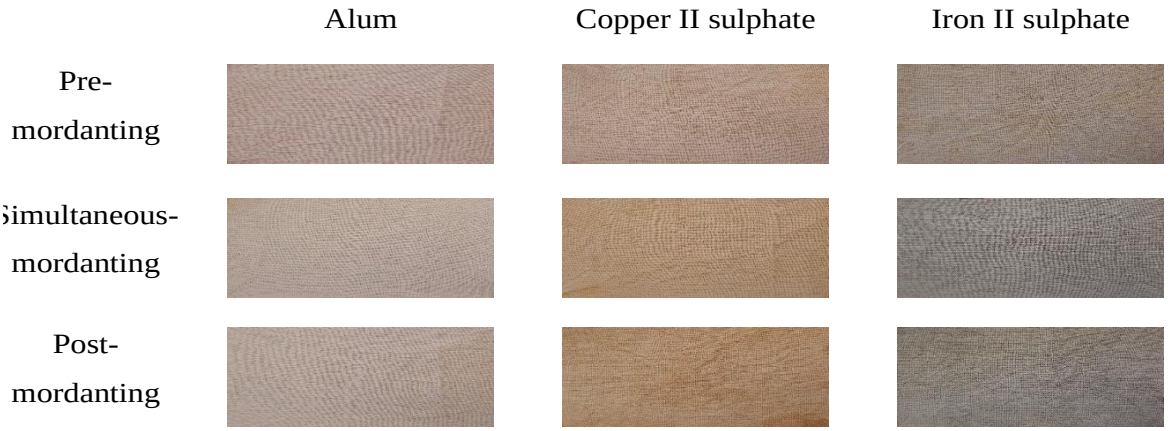


Figure.4 Color of cotton fabric after dyeing by (i) Pre-mordanting (ii) Simultaneous-mordanting (iii) Post-mordanting method

According to the result, the color of cotton cloth dyeing with water extract of pre, simultaneous and post-mordanting methods of Babul (Sha) was the highest color form.

Table 3. Effect of Mordant on Changes in Color Fastness of Cotton Fabric Dyed with Babul Barks using Pre-mordanting, Post-mordanting and Simultaneous-mordanting Methods

No	Types of Mordant	Sample Name	Rubbing Fastness		Washing Fastness		
			Dry	Wet	Change of Shade	Staining on	
						cotton	p/c
1	Pre-mordanting	Alum	4	3-4	1	4	4
		$CuSO_4$	4	3	1	4	4
		$FeSO_4$	4	3	1	4	4
2	Post-mordanting	Alum	4	3-4	1	4	4
		$CuSO_4$	4	3-4	1	4	4
		$FeSO_4$	4	3	1	4	4
3	Simultaneous mordanting	Alum	4	3-4	1	4	4
		$CuSO_4$	4	3	1	4	4
		$FeSO_4$	4	3	1	4	4

1 = very poor; 2 = poor; 3 = fair; 4 = good; 5 = excellent; p/c = polyester + cotton

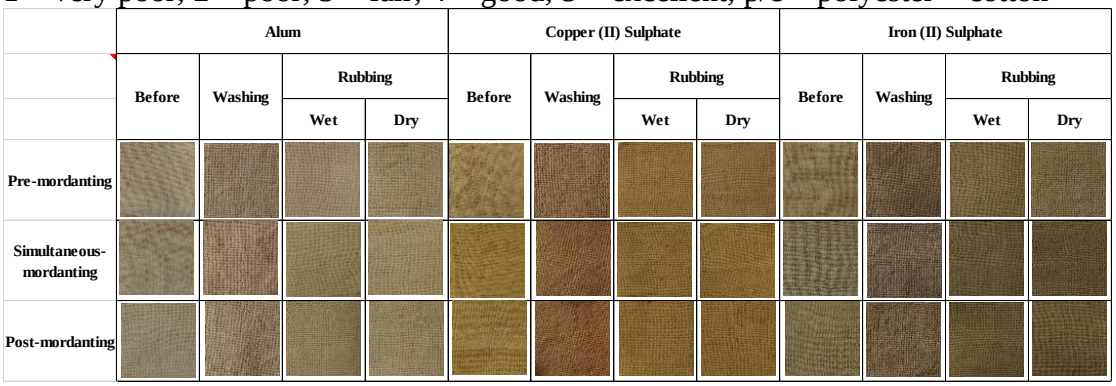


Figure.5 Fastness color result of cotton fabric dyeing with water extract by Pre, Simultaneous, and Post-mordanting methods of Babul (Sha)

Testing the Color Fastness of Dyes fabric

Good dry rubbing fastness was found in cotton fabric dyed by alum, $CuSO_4$, and $FeSO_4$ mordant. In washing fastness, the change of shade ratings showed poor fastness whereas staining on cotton and polyester/cotton fabric had a good fastness grade. For the changing shade in the washing fastness test, the alum mordant gave comparatively better fastness than the $CuSO_4$ and $FeSO_4$ mordants used. The post-mordanting method of the Babul (Sha) sample with alum and $CuSO_4$ mordant were due to the better results of color fastness than that of the pre-mordanting and simultaneous mordanting. Therefore, the post-mordanting method with alum and $CuSO_4$ mordant were the most suitable for natural dye extracted from the bark of the Babul (Sha).

Conclusion

A phytochemical investigation of the sample revealed the presence or absence of phytochemical constituents such as alkaloids, flavonoids, reduction sugars, terpenes, glycosides, steroids, phenolic compounds, tannins, and saponins. In the antimicrobial activity tests, the water and ethanol extracts of the sample showed the highest activity on *Candida albicans, Pseudomonas fluorescens, and Staphylococcus aureus*. Therefore, the dye solution can be used safely for human skin and also gives a cooling effect to the skin even in hot summer. From the result of the FT IR spectrum, it can be said that the extracted dye contained the hydroxyl group and aromatic ring with the ether linkage which are the characteristics of tannin compounds. In this research, three mordants were used and the alum gave the significant color because alum mordants are known for their ability to form coordination complexes and to chelate with dye and interact with the fibers. In the three mordanting methods, the post-mordanting method gave the attractive color than the two other methods. In the dyeing process rubbing fastness properties of dye cotton were acceptable of 4 (good) for the dry mark and 3,3-4 (fair, good) and wet mark for all with three mordants. Moreover, the washing fastness properties of dyed cotton were the acceptable value of 1 (very poor) in shade and 4 (good) in cotton respectively. Therefore, dyed cotton with different mordants method and mordanting methods had better washing fastness and rubbing fastness properties in the dyeing process.

Acknowledgment

The first author is greatly indebted to Dr Aye Aye Han, Rector and Dr Kyaw Win Oo and Dr Myint Myint Khaing Pro-rectors, for their permission to present the research. I would like to express our thanks to Dr Kyae Mon Lwin, Professor and Head, Dr Daw Tin Sein, Professor, of the Department of Chemistry, at Myingyan University for their encouragement to present this paper.

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