

OPTIMIZATION OF TANNIN EXTRACTION FROM TAMARIND SEED TESTA AND ITS APPLICATION

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Abstract

Tamarind (*Tamarindus indica* L.) is abundantly found in Myanmar and is also a commercially used plant. In natural dyeing tannins play an important role as a mordant and have an affinity to cellulose as well as to protein fiber. This study is aimed to the extraction of natural tannin from tamarind seed testa and application of cotton and silk fabric as natural mordant. The color compounds present in tamarind seed testa were identified by phytochemical tests. The mineral content of tamarind seed testa was analyzed by Energy Dispersive X-ray Fluorescence (EDXRF) spectroscopy. Tannin was extracted from tamarind seed testa using ethanol-water mixture by simple distillation method. The effects of extraction temperature, time and ethanol-water ratio on the yield percent of tannin powder were investigated. In the extraction with 95 % ethanol-water mixture, ethanol-water ratio (35:15), extraction time 60 min and temperature 50 °C were suitable conditions for the preparation of extracted tannin. The functional groups present in extracted tannin was determined by using FTIR spectrometer. The extracted tannin powder was applied as a natural mordant alone and the tannin treated fabric was further mordanted with copper sulphate for dyeing of cotton and silk fabrics. The washing and rubbing fastness of dyed fabrics were tested by grey scale.

Keywords: tamarind seed, simple distillation, tannin, mordant, fastness

Introduction

Tannins are polyphenolic compound that bind and precipitate proteins, amino acids and alkaloids. Tannins are present in almost every part of plants, such as leaves, barks, wood, roots, fruits, and seeds. They are widely used in the production of cationic dyes, ink, clarifying agents in wine, textile dyes, fruit juice antioxidants, and coagulants in the production of rubber (Khanbabaee and Van Ree, 2001). They are able to combine with various substances and precipitate in aqueous solution (Lokeswari and Sujatha, 2011). In natural dyeing they take part an important role as a mordant. They are easy to extract with warm water and have an affinity to cellulose as well as to protein fiber. Tannins can also form complexes with metal ions such as copper, lead, zinc, cadmium, and manganese, which are insoluble compounds. Formation of the tannin complexes with metal compound is important in the mordanting of cellulose fiber. Tannic acid is also a specific commercial form of tannin.

Tamarind tree (*Tamarindus indica* L.) trees are abundant in Africa and Southeast Asia. These trees contain several brown pods with dark brown seeds bounded by a gummy pulp that dries naturally to a sticky paste. Tamarind fruits have outer-shell, pulp and the seeds. The seeds have about 25 % testa and 75 % kernel. Testa is of brown colour and kernel is of light cream. The seeds are used in the textile industry as a sizing substance as well as a source of polysaccharide, glue oil, and tannin for different industrial applications. They are primarily used in the preservation of leather glues, stains and mordants (Swarna et al., 2009). Meanwhile, some of the natural fibres such as cotton have very low affinity for most of the natural dyes. The tannins serve as an important role in cotton dyeing to retain colouring matter permanently. The ultimate aim, the purpose of preparing the natural fibers with tannin is not so much to fix the colouring matter, as to fix certain metallic salts such as copper, iron, potassium etc., in the form of insoluble tannates. The metal tannates present on the material forms insoluble lakes with the natural dyes during the dyeing process and results in improved fastness properties (Gulrajani, 1992).

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In textiles dyeing industry, most of the dyes employed are the synthetic azo dyes of which the azo bond cleavage brings about aromatic amines. These synthetic azo dyes may be harmful to human health and environment although they can greatly reduce the cost and enhance certain application and wear characteristics. For these reason, natural dyes are among the promising options for the waste. After the dyeing process, they are easily absorbed by the soil (Seiko Akiyama, 1996). The main objective of the mordant when used with adjective dye is to open up the pores so that the dye can penetrate the fibres, thereby supporting in the fixation of the dyestuffs on the substrate. However, mordants can also be used with dyes which may be applied directly to the fiber. In this case the function of the mordant is to form an insoluble compound with the dyestuff within the fiber itself, thereby improving the fastness properties of the dyed material (Patel, 2011).

The objective of this work was to extract tannin from tamarind seed testa. The specific objectives of this study were to study the suitable extraction conditions by using different extraction temperatures, time and ethanol-water ratios on yield percent of tannin powder, to study the absorbance value of extracted tannin solution by UV spectrophotometer and to evaluate the fastness properties of dyed cotton and silk fabrics.

Materials and Methods

Materials

Tamarind seeds were collected from Nyaung Oo Township, Mandalay Region. The required chemicals for processing were purchased from Able Teaching Aids, at the corner of 35th street and 85th street, Maha Aung Mye Township, Mandalay Region. Cotton and silk fabrics were purchased from the Saunders Weaving and Vocational Institute (SWVI), Amarapura Township, Mandalay Region.

Methods

Preparation of Tamarind Seed Testa Powder

The tamarind seeds were rinsed with water to remove impurities. The seeds were dried in hot air oven at 120 °C for 45 min and the testa were removed by crushing and then grinding into powder form. The tamarind seed testa powder was sieved through a 40-mesh screen and stored in an airtight container at room temperature for further use.

Phytochemical Screening of Tamarind Seed Testa

Before the extraction of natural dyestuff, phytochemical investigation was carried out to study the organic compounds which are present or absent in the tamarind seed testa. The experiments were conducted according to the procedures mentioned in “A Guide to Modern Techniques of Plant Analysis” (Harbone, 1984).

Determination of Mineral Contents of Tamarind Seed Testa

The quantitative analysis of tamarind seed testa was carried out by using Shimadzu EDX-700 spectrometer, Japan. The resultant EDXRF spectrum is illustrated in Figure 1 and the relative abundance of the elements found in tamarind seed testa are described in Table 2. EDXRF analysis was carried out at Laboratory of Chemistry Department, University of Mandalay.

Extraction of Tamarind Seed Testa Tannin using Ethanol-Water Mixture

Tamarind seed testa powder 5 g, 35 mL of ethanol and 15 mL of water were added to a 500 mL 3-neck round bottom flask. The round bottomed flask was fitted with condenser and the extraction was carried out at 50 °C for 60 min. Afterwards, the extract solution 50 mL was cooled and filtered through a filter paper and collected. The residue was extracted again with 50 mL of the same ratio of ethanol -water mixture at the same temperature and time. The filtrate 100 mL was cooled, filtered again and ethanol was removed by distillation. The total extract was cooled and tannin was separated with petroleum ether by using a separating funnel. The maximum absorbance value (optical density) of a tannin extract was measured by UV-Vis spectrophotometer (Model-PD-303S, APEL) at a wavelength of 725 nm (Elgailani and Ishak, 2014). Then the extracted tannin solution was concentrated and dried at 60°C for 6 h in an oven.

The experiments using the same procedures as mentioned above were conducted by using different extraction temperatures (40 °C, 60 °C, 70 °C), extraction time (30 min, 90 min, 120 min) and ethanol-water ratios (15:35, 25:25, 45:5).

Identification of Extracted Tamarind Seed Testa Tannin by FT-IR Spectrophotometer

The chemical characteristic profiles and identification of functional groups of the tamarind seed testa tannin was determined by SHIMADZU FT-IR spectrophotometer.

Qualitative Analysis of Tamarind Seed Testa Tannin

The qualitative analysis was carried out to find the class of tannin obtained from tamarind seed testa. The experiments were carried out by treating 0.5 % solution of the extracted tannin with various reagents such as 2 % gelatin, 5 % aqueous ferric chloride, 10 % lead acetate, copper sulphate solution, dilute hydrochloric acid solution and dilute sulfuric acid solution. The change in color of the tannin solution was observed after the addition of the reagents.

Dyeing Process of Fabrics using Coconut Husk

Preparation of Coconut Husk Dye Liquid

For the preparation of coconut husk extract, 30 g of coconut husk powder (mesh no. 20) and 1200 mL of water were mixed in a stainless-steel pot and heated at 90 °C for about 45 min resulting in a final dye solution volume of 600 mL. After that, the dye solution was cooled, filtered and stored at room temperature for dyeing of cotton and silk fabrics.

Mercerization of Cotton Fabric

Mercerization was done according to the standard procedures (Seiko Akiyama, 1992). To prepare mercerized cotton fabrics, 1 yard of cotton (about 100 g) fabric was scoured using a solution of 5 g caustic soda in two litres of warm water. And then, the cotton fabrics was simmered in this solution for 1 h. After completion of this step, the fabric was put into vinegar solution (4 mL acetic acid to one litre of water) to neutralize it. Then, the fabric was rinsed with water and then dried at room temperature.

Mordanting of Cotton Fabrics by Pre-Mordanting Method

In this method, mordant solution was prepared by heating 1 g (0.5 % w/v) of tamarind seed testa tannin with 200 mL of water. This solution was heated to a temperature of 80 °C in a water bath and 7 g (25 cm × 25 cm) of cleaned fabric was simmered in solution for about 45 min. During mordanting, the fabric was frequently stirred with a glass rod to obtain good penetration of mordant into the cotton fabric. Then the mordanted cotton fabric was rinsed with water and allowed to air dry.

The other mordant solution was prepared by heating 0.1 g (0.05 % w/v) of copper sulphate with 200 mL of water and the tannin treated fabrics were simmered in this solution for about 45 min. The remaining procedures were carried out as mentioned above.

Dyeing of Mordanted Cotton Fabrics

After mordanting, 200 mL of prepared concentrated dye was heated at 80°C and mordanted fabric was simmered in this solution for 60 min. After that, the dyed fabric was rinsed with water and allowed to air dry. The colour developed on dyed cotton fabrics are shown in Figure 4.

Dyeing Process of Silk Fabrics

In this method, 3 g (25 cm × 25 cm) of cleaned silk fabric was simmered in 200 mL of warm water for about 1 h. After that, mordant solution was prepared by heating 1 g of the tamarind seed testa tannin with 200 mL of water. The remaining procedures were the same as mentioned in mordanting of cotton fabrics. The dyeing procedures were the same as mentioned above. The colour developed on dyed silk fabrics are shown in Figure 4.

Testing the Colour Fastness of Dyed Fabrics

After the dyeing process, testing the colour fastness of dyed fabrics such as washing fastness and rubbing fastness were determined.

Washing Fastness

Lauder Meter washing machine, Model L-4 (containing 4-rack testing bottle) washing machine was used and Test No. 2 of ISO 105 was used to assess the colour fastness to washing. 2 g/L of soap solution was added to the jar (material to liquor ratio of 1:50) and the soap solution was preheated to 50°C. Then the composite sample was placed in the jar and treated at 50 °C for 45 min. After washing, the fabrics were rinsed with water for 3 times and dried at room temperature.

Rubbing Fastness

The rubbing fastness of dyed fabric was determined by JIS STD L-0241 Rubbing Tester Machine. The test specimen (25 cm × 5 cm) and a piece of white bleached cotton fabric about (5 cm × 5 cm) were cut parallel to the warp direction for the rubbing fastness test. In dry rubbing fastness test, the test specimen was mounted on the white bleached cotton cloth and placed on the rubbing tester. The test specimen was rubbed with 500 g load at the speed of 30 circles per minute for about 100 times.

In this study, both dry and wet rubbing fastness tests were carried out. In wet rubbing fastness test, the rubbed fabric was wetted with water and the amount of water was two times of the fabric weight. The fabric was finally dried at room temperature. The color stained on bleached cotton fabrics were compared by grey scale for staining and the degree of fastness rating was determined.

Results and Discussion

Table 1 shows the results of phytochemical screening of tamarind seed testa. According to the results obtained from this test, it can be seen that the colour compounds such as phenolic, tannins, flavonoids, glycosides, saponins, alkaloids and polyphenols are present in the tamarind seed testa.

Table 1: Phytochemical Screening of Tamarind Seed Testa

Sr. No.	Tests	Extract	Reagents	Observation	Inference
1.	Phenolics	Distilled Water	1 % FeCl ₃	Violet Color	+
2.	Tannins	Distilled Water	1 % FeCl ₃	Deep Blue Precipitate	+
3.	Flavonoids	95 % Ethanol	Conc: HCl + Mg	Pink Color	+
4.	Glycosides	Distilled Water	10 % FeCl ₃	Purple colour	+
5.	Saponins	Distilled Water	NaHCO ₃	Frothing	+
6.	Alkaloids	1 % HCl	Dragendroff's Reagent	Orange Precipitate	+
7.	Polyphenols	95 % Ethanol	1 % FeCl ₃ + 1 % K ₃ Fe(CN) ₆	Greenish Blue Color	+

Note: (+) = present, (-) = absent

According to the results of Table 2 and Figure 1, the elements found from tamarind seed testa powder were calcium, potassium, sulphur, aluminium, silicon, phosphorus and chlorine. Among them, calcium is the most abundant element and contains 0.544 %.

Table 2: Relative Abundance of Elements in Tamarind Seed Testa

Sr. No.	Symbols	Elements	Concentration (%)
1.	Ca	Calcium	0.544
2.	K	Potassium	0.0820
3.	S	Sulphur	0.0678
4.	Al	Aluminium	0.0666
5.	Si	Silicon	0.0471
6.	P	Phosphorus	0.0283
7.	Cl	Chlorine	0.0263

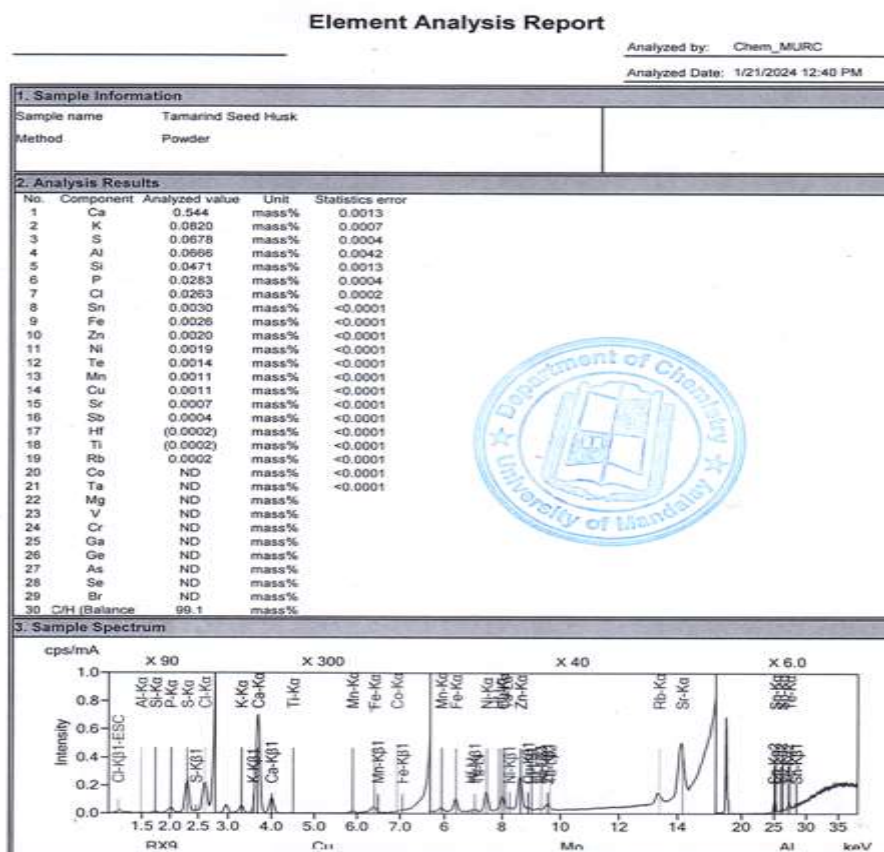


Figure 1: EDXRF Spectrum of Tamarind Seed Testa Powder

According to the Beer-Lambert law, absorbance value is directly proportional to the concentration of solution and the extraction of tannin solution at 50°C for 60 min was more suitable than the other conditions because of the maximum absorbance value and yield percent of 0.3344 and 33.6 % at 725 nm. The results are shown in Table 3. It can also be clearly seen that the increase in extraction temperature above 50 °C gave decrease in absorbance value and yield percent of tannin powder.

Table 3: Effect of Extraction Temperature on the Absorbance Value and Yield Percent of Tamarind Seed Testa Tannin

Weight of Tamarind Seed Testa Powder = 5 g

Ethanol: Water = 25 : 25

Wavelength = 725 nm

Sr. No.	Extraction Temp. (°C)	Extraction Time (min)	Volume of Solvent (mL)	Absorbance Values	Extracted Powder (g)	Yield % (w/w)
1.	40	60	50	0.16	1.18	23.6
2.	50*	60	50	0.334	1.68	33.6
3.	60	60	50	0.169	1.67	33.4
4.	70	60	50	0.180	1.4	28

*The most suitable condition

According to the results of Table 4, maximum absorbance value and yield percent of tannin powder was observed with 60 min extraction time but after that the absorbance value was decreased from 0.334 to 0.176.

Table 4: Effect of Extraction Time on the Absorbance Value and Yield Percent of of Tamarind Seed Testa Tannin

Weight of Tamarind Seed Testa Powder = 5 g

Ethanol: Water = 25 : 25

Wavelength = 725 nm

Sr. No.	Extraction Time (min)	Extraction Temperature (°C)	Volume of Solvent (mL)	Absorbance Values	Extracted Powder (g)	Yield % (w/w)
1.	30	50	50	0.168	0.94	18.8
2.	60*	50	50	0.334	1.67	33.4
3.	90	50	50	0.224	1.42	28.4
4.	120	50	50	0.176	1.39	27.8

*The most suitable condition

Table 5 tabulates the effect of ethanol- water ratio on maximum absorbance value and yield percent of tannin powder. According to the results, it was clearly seen that ethanol: water ratio of 35:15 was more suitable than other conditions because of the absorbance value and yield percent of 1.7 and 34 %.

Table 5: Effect of Ethanol -Water Ratio on the Absorbance Value and Yield Percent of Tamarind Seed Testa Tannin

Weight of Tamarind Seed Testa Powder = 5 g

Wavelength = 725 nm

Sr. No.	Solvent Ratio	Extraction Time (min)	Extraction Temp. (°C)	Absorbance Values	Extracted Powder (g)	Yield % (w/w)
	Ethanol: Water					
1.	15 : 35	60	50	0.212	1.43	28.6
2.	25: 25	60	50	0.334	1.67	33.4
3.	35: 15*	60	50	0.601	1.7	34
4.	45: 5	60	50	0.156	1.56	31.2

*The most suitable condition



Figure 2: Tamarind Seed Testa Tannin

Figure 3 shows the FT-IR spectrum of tamarind seed testa tannin powder extracted by 95 % ethanol-water mixture. Stretching band of alkenes group was found at 771.53 cm^{-1} . Alcohol and phenol group gave the absorption band at 1249.87 cm^{-1} , 1203.58 cm^{-1} , 1103.28 cm^{-1} and 1056.99 cm^{-1} . The absorption bands at 1280.73 cm^{-1} and 1365.60 cm^{-1} was attributed to CH symmetric bending vibration. CH asymmetric bending vibration was found at 1442.75 cm^{-1} . The band at 1519.91 cm^{-1} and 1604.77 cm^{-1} were characteristics of stretching region of aromatic groups. The peak at 1604.77 cm^{-1} showed the characteristics of condensed tannin and these values were in agreement with the range of literature value reported by (Sirisangsawang and Phetyim, 2023). The stretching vibration of carbonyl group was found at 1732.08 cm^{-1} . In addition, absorption band at 3356.14 cm^{-1} , 3317.56 cm^{-1} and 3232.70 cm^{-1} indicated the presence of amines group.

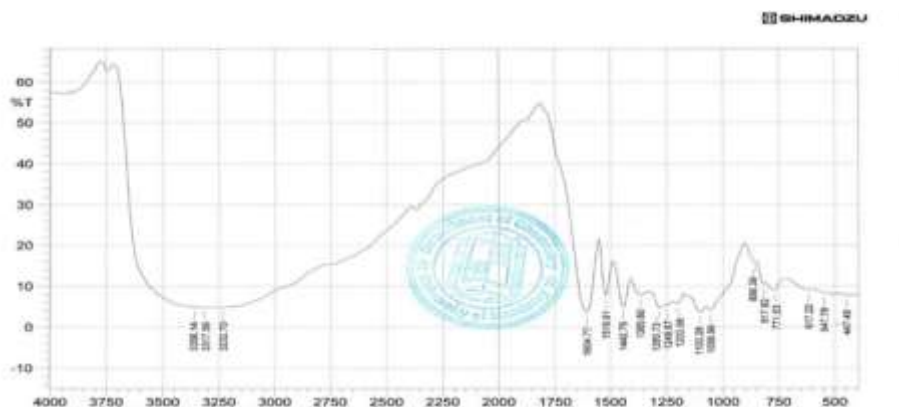


Figure 3: FTIR Spectrum of Tamarind Seed Testa Tannin

From the results of Table 6, the tamarind seed testa gave identical colour and precipitation reaction when compared with the phlobatannins or condensed tannins as mentioned in the literature (Russell, 1935). It produced a dark green precipitate with aqueous ferric chloride and dark red solids with dilute HCl solution. It clearly confirms that tamarind seed testa extract is of condensed tannins.

Table 6: Qualitative Analysis of Tamarind Seed Testa Tannin

Sr. No.	Reagents	Observations
1.	10 % Lead acetate	Pinkish precipitate
2.	2 % Gelatin	Dirty white precipitate
3.	5 % Aqueous Ferric chloride	Dark green precipitate
4.	10 % CuSO ₄ solution	Faint green
5.	HCl (dil)	Dark red solids
6.	H ₂ SO ₄ (dil)	Flesh-coloured precipitate

The characteristics of tamarind seed testa tannin are shown in Table 7. According to the results, the moisture content extracted by ethanol-water mixture are moisture content 7.6 %, total solids 92.4 % and pH 5.11.

Table 7: Characteristics of Tamarind Seed Testa Tannin

Sr. No.	Characteristics	Prepared Sample	Literature Value*
1.	Moisture Content, % (w/w)	7.6	8.40
2.	Total Solids Content, % (w/w)	92.4	91.60
3.	pH	5.11	4.57

* Nyaga et al., 2023

From the results of Table 8, cotton fabrics mordanting with 0.5 % tamarind seed testa tannin was found to be more suitable due to its good wet rubbing fastness grade of 4. According to the results of silk fabrics, it was demonstrated that mordanting with 0.5 % tamarind seed testa tannin gave very poor in change of shade mark than other conditions. It was also found that mordanting with tannin gave poor fastness in change of shade mark and dark brown colour on dyed fabrics mordanting with tamarind seed testa tannin and CuSO_4 .

Table 8: Effect of Mordants on Color Fastness of Fabrics Dyeing by Pre-Mordanting Method

Coconut Husk Dye Powder	= 10 g
Mercerized Fabric	= 25 cm × 25 cm
Mordanting Temperature and Time	= 80 °C, 45 min
Dyeing Temperature and Time	= 80 °C, 60 min
Volume of Dye Solution	= 200 mL

Sr. No.	Sample Code	Washing Fastness (60 °C, 30 min)			Rubbing Fastness (500 g, 100 Times)	
		Change of Shade	Staining on Cotton		Dry	Wet
			Cotton	p/c		
1.	*C ₁	2	4	4	4	4
2.	C ₂	2	4	4	4	3
3.	S ₁	1	4	4	4	4
4.	*S ₂	2	4	4	4	4

*The most suitable condition

Notes: C₁ = Dyed Cotton fabrics Mordanting with 0.5 % Tamarind seed testa tannin

C₂ = Dyed Cotton fabrics Mordanting with 0.5 % Tamarind seed testa tannin and 0.05% CuSO_4

S₁ = Dyed Silk fabrics Mordanting with 0.5 % Tamarind seed testa tannin

S₂ = Dyed Silk fabrics Mordanting with 0.5 % Tamarind seed testa tannin and 0.05% CuSO_4

5 = excellent, 4 = good, 3 = fair, 2 = poor, 1 = very poor, p/c = polyester + cotton (Lye, Siegert, 1977)

Sr. No.	Sample Code	Original Sample	Washing Fastness (I.S.O Test No.2) (50 °C, 45 min)	Rubbing Fastness (500 g, 100 Times)	
				Dry mark	Wet mark
1.	C ₁				
2.	C ₂				
3.	S ₁				
4.	S ₂				

Figure 4: Colour Fastness of Fabrics Dyeing with Coconut Husk

Conclusion

In this research work, natural tannin was extracted from tamarind seed testa using ethanol due to its polar nature, which allows tannin to effectively dissolve, and it is also a safe and relatively inexpensive solvent. According to the results of phytochemical tests, it was found that the colour compounds such as phenolic compounds, tannins, flavonoids, glycosides, saponins, alkaloids and polyphenols were present in tamarind seed testa. From the results of EDXRF analysis, the relative abundance is found to be 0.544% Ca, 0.0820 % K, 0.0678 % S, 0.0666% Al, 0.0471 % Si, 0.0283 % P and 0.0263 % Cl. In addition, the most suitable conditions for extraction of tannin from tamarind seed testa were ethanol-water ratio (35:15), extraction temperature 50°C and extraction time 60 min according to the results of absorbance value 0.601 and yield percent of 34.

It was evident that prepared tamarind seed testa tannin is of condensed tannins according to the qualitative analysis and the results of FTIR analysis. The cotton fabrics dyeing by pre-mordanting method with 0.5 % tannin gave attractive colour and acceptable wet rubbing fastness

of 4 (Good). It was observed that the fastness results of silk fabric mordanting with 0.5 % tannin and 0.05% copper sulfate was found to be more suitable when compared with tannin alone.

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