



# Test Protocol for Identifying Natural Rubia (Madder) as a Natural Dye from Eco-Friendly Natural Fibre Based Natural Dyed and Finished Oeko-Tech Textiles Differentiating Selective Natural Dye from its Synthetic Counterpart/Analogue

Ashis Kumar Samanta<sup>1\*</sup>, Padma S Vankar<sup>2</sup>, Dhara Shukla<sup>2</sup>, YA Shah<sup>3</sup>, Md Irfan<sup>3</sup> and Nilendu Sekhar Bhaumik<sup>1</sup>

<sup>1</sup>Department of Jute and Fibre Technology, University of Calcutta, India

<sup>2</sup>FEAT, India

<sup>3</sup>Ama Herbals Laboratories Pvt Ltd., India

\*Corresponding author: Ashis Kumar Samanta, Department of Jute and Fibre Technology, University of Calcutta, 35, B C Road, Kolkata-700 019, West Bengal, India

submission: 📅 April 04, 2018; Published: 📅 April 19, 2018

## Abstract

In this paper, test protocol to identify Madder/Manjistha extracted colourants as a natural dye has been developed and established for Differentiating it from its synthetic counterpart synthetic alizarin. This test methods by various testing like TLC, HPLC and also by UV-VIS Spectrophotometry with high level of accuracy and confirmation. This will benefit the producer and consumers to identify originality of using natural colorant in dyed textiles to make it ecofriendly and also for reduction of Pollution load in the environment.

## Introduction

The revival of Natural dyes has been for two reasons, i) Due to the harmful effect of synthetic dyes globally safer natural dyes are being preferred. ii) Ban of Azo dyes has also brought back natural dyes in limelight. Natural dyes are being used extensively for dyeing and printing of cotton, silk and wool substrate. Thus Natural fibre based textiles dyed/printed and finished with natural agents are special category of Oeko Tech Textiles reducing pollution load to a significant extent.

Rubia (Maddar) or Manjistha as a natural Dye has its own characteristics of being a natural dyestuff and is botanically known as Rubiacordifolia, which produces anthraquinone based reddish orange colour in its roots, stem and leaves, which are being used worldwide for dyeing and printing natural fibre based textiles since ancient times. It grows throughout India, in hilly districts. Generally this plant is found on the Himalayan side of India. It is a perennial, herbaceous climber. It is a good source of anthraquinone and alizarin based reddish orange dyes which is obtained from roots, stem and leaves of the plant. Earlier literature review on some important work and review on the same filed [1-11] indicate

that such approach has not been initiated so far and need in depth analysis.

This is a non dischargeable natural dyestuff, but is suitable for direct printing as well. Structurally the Rubia (Madder or manjistha) natural dye consists of natural alizarin, manjistin, purpurin and other anthraquinone moieties as colour component.

Similarly Rubiatinctoria (European Maddar) is another species of Rubia or madder natural dye, which also belongs to anthraquinone based dyes and has similar color content.

So a test protocol for identifying natural indigo has been developed jointly by FEAT –IIT-Kanpur and DJFT, IJT, CU under initiation and industrial partnership of M/S Ama Herbals Lab. Pvt Ltd, Lucknow under Govt. Funding From Ministry of Textiles, Govt. of India. Through Textile Commissioner's office, Govt. of India.

The main intention of the present research work was to identify the Rubia and Indigo as natural dyestuff from their synthetic analogue which is usually used as adulterant for cost reduction and color enhancement purposes. Brief test protocol is discussed

below for creating awareness amongst consumers for prevention of adulterants.

### Test protocol developed for identification of rubia ( madder ) as natural dye from its dyed textiles

**Extraction and purification process:** The pale orange stem pieces of *Rubia cardio folia* were ground into powder which was sources from himayalyan region of India (also avialbale in Nepal and Sri Lanka). A crude extract was initially thus prepared. The dried-ground powder (30g) was soaked in sufficient water (150mL) at 70-°C for 30min. After extraction, the extract was filtered through ordinary filter paper and the filtrate was collected, and absorbance was recorded in UV-VIS spectrophotometer for the determination of concentration. Condition of extraction used was Mass to Liquor ratio: 30g in 150mL, Temperature at 70 °C and time for 1.5hr. Finally the extracted dye was purified by vaccum evaporation and Soxhleting for 10 cycles by using alcohol: toluene mixture and washing in acetone.

**Application process:** Cotton fabric scoured by the usual method is further treated with 5 % Tannic acid and 10% natural potash alum mordant at 80 °C for 30min. Dyeing of the alum pre mordanted cotton fabric was done at two different pH 5 and 13, with 4 % purified madder extract to check the best and optimum condition for maximum colour strength/hues obtained. The experimental dyeing conditions used was 1:20 Material to Liquor ratio, temperature at 80 °C for 30min using 4 % (OWF) madder purified dye powder.10gpl of sodium chloride solution was also added to the dye bath for better exhaustion of dye .

**Table 1:** Colour fastness properties.

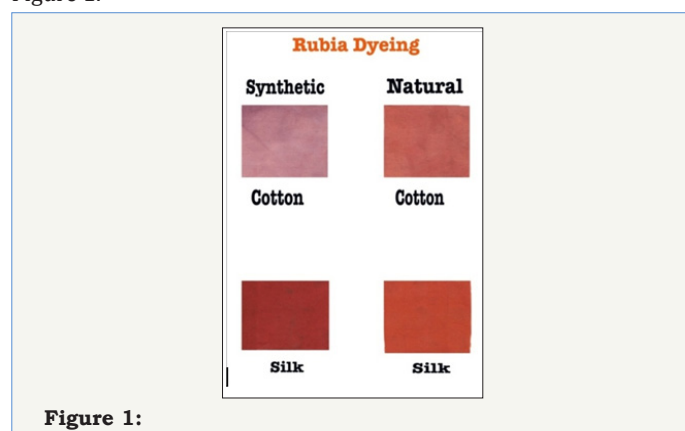
S. No	Parameter	Requirement (Minimum)		Test Method
		Natural <i>Rubia</i>	Alizarin	
1	Colour fastness to washing	4-5	2-3	IS/ISO 105 C10 Color fastness to washing with soap or soap and soda
	-Change in colour -Staining on cotton	5	4	
2	Colour fastness to light	4-5	4	IS 2454-1985
3	Colour fastness to Perspiration			IS 971: 1983
	Acidic:	3	2-3	
	-Change in colour	4-5	4-5	
	-Staining on cotton	3	3	
	-Alkaline	4-5	3	
	-Change in colour			
	-Staining on cotton			
4	Colour fastness to Rubbing			IS 766: 1988
	-Dry	4-5	4	
	-Wet	4-5	4	

### Dyeing swatches and their colour fastness results

Shades obtained from Natural Rubia dye on cotton fabric are shown in colour Figure 1 given below:

#### Color fastness properties of fabric swatches dyed with natural rubia

The indicative color fastness rating of the dye on cotton fabric dyed by the modified method prescribed, it shall satisfy the requirements given in Table 1 as corresponding for the range of color fastness is given in Table 2. The specific tests were: color fastness for light, IS-2454-1985 by xenotester, color fastness to rubbing, IS-766-1988 by crock meter, color fastness to washing; and IS/ISO 105 C10 by wash wheel, color fastness to Perspiration, IS-971-1983 by Perspirometer. Shade cards are shown in colour Figure 1.



**Figure 1:**

**Table 2:** Observation from chromatogram of natural *Rubia* and synthetic alizarin.

Peak Reading Received At (Nm)	In Synthetic Alizarin	In Natural <i>Rubia</i>	Comment (Describing Difference With Reason)
255nm	One Main Peak is at 1.6mins and no Peak after 3.5mins	1.8mins main Peak, small Peaks at 2.5 and 11.3mins	One Main Peak is at 1.6mins and no Peak after 3.5mins while Natural <i>Rubia</i> has main Peak at 1.8mins and a characteristic Peak at 11.3mins

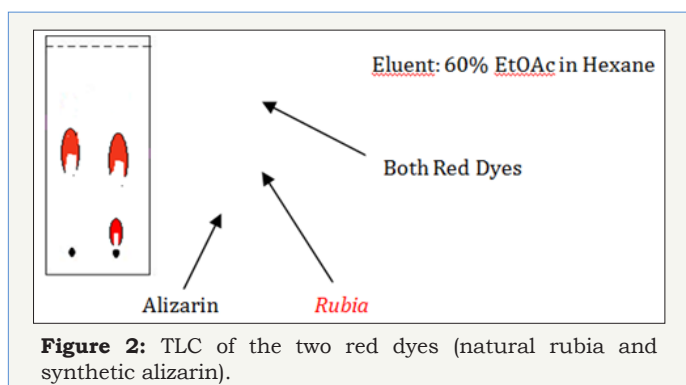
### Characterization and standardization of rubia as natural dye for identification of rubia (madder) from its dyed textiles

**Method-1: Thin Layer Chromatography (Preliminary Test/ Indicative Test):** TLC is also used to support the identity of a compound in a mixture when the  $R_f$  of a compound is compared with the  $R_f$  of a known compound (Preferably both run on the same TLC Plate). Natural *Rubia* dye and synthetic alizarin dyes are compared on TLC to identify natural rubia containing manjisthin as one of the main colour component.

**A. TLC test procedure for rubia dye:** Rubia dye sample is prepared (0.01gm of madder sample in 20ml of solvent) is dissolved in solvents such as ethyl acetate or dichloromethane.

**Table 3:** TLC plate observation.

$R_f$ On The TLC Plate	In Synthetic Alizarin	In Natural <i>Rubia</i>	Comment (Describing Difference With Reason)
60% EtOAc in Hexane	$R_f$ 0.60	$R_f$ 0.60 and 0.20	Presence of two spots in <i>Rubia</i> is because of alizarin and Purpurin



**C. Conclusion from TLC test:** Color of the spot and  $R_f$  values of natural *Rubia* (RC) and synthetic red (Alizarin) are very different, *Rubia* showing one spot which is fairly Polar as shown in Figure 2.

### Method 2: Identification of Natural Rubia dye with Synthetic Alizarin by HPLC method ( one of the confirmatory Test):

a) Identification of compounds by HPLC is a crucial Part of any HPLC assay. In order to identify any compound by HPLC a detector must first be selected. UV detector was selected and was set to optimal detection settings, a separation assay was developed. The parameters of this assay were made to be such

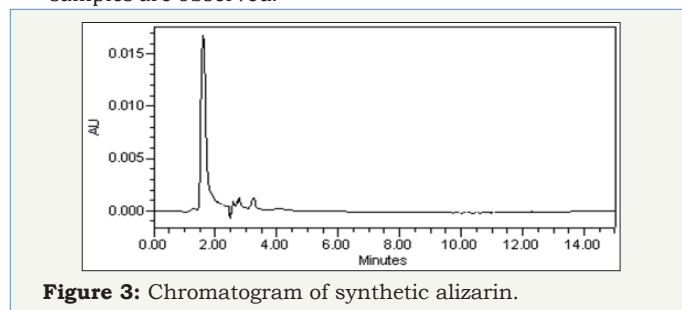
The prepared sample is agitated in sonicator, a mechanical agitating device used for dissolution of madder dye molecules) for 5min for complete dissolution of the *Rubia* purified dye powder sample. The solution is then spotted on silica gel coated plate for elution. TLC Plates were eluted using 60% Ethyl acetate (EtOAc) /40% Hexane as suitable fluent system for such red/orange dyes. The spots were visualized in visible light as well as in iodine chamber. The possible constituents of the extracts are identified by comparing TLC data, i.e. color of the spot and  $R_f$  values of natural *Rubia* containing manjisthin and synthetic alizarin dye.

### B. TLC plate observation

Table 3

that a clean peak of the both the samples are observed from the chromatograph.

b) The two red dye (*Rubia* containing manjisthin and synthetic alizarin) samples were weighed separately (0.1gm) in 1000ml of methanol and 1 $\mu$ L of the prepared solution was injected to the C-18 reverse phase column and eluted through column. The base line showed response within a run of 15mins for natural *Rubia* and synthetic alizarin. The parameters of this assay were made to be such that a clean peak of the both the samples are observed.



c) Clear observation was made from the two red dye samples as can be seen in the two chromatographs given below (Figure 3 & 4). For Synthetic Alizarin, One main Peak is at 1.6mins and no Peak after 3.5mins, while Natural *Rubia* has main peak at 1.8mins and a characteristic peak at 11.3mins (Table 2).

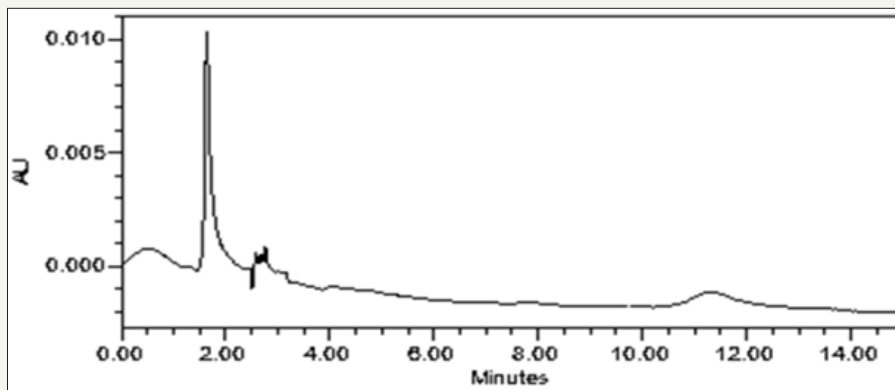


Figure 4: Chromatogram of natural rubia.

d) Conclusion of HPLC test for differentiating rubia from synthetic alizarin: Natural Rubia and synthetic alizarin show clear differences in their characteristic peaks and their retention times and their peak heights. Natural Rubia will always be showing two equivalent peaks for as compared to synthetic alizarin which shows three peaks due to unpurified sample of the latter, this is a mark of identification between natural Rubia and synthetic alizarin.

**Method-3: Identification of natural rubia dye with synthetic alizarin by UV-vis spectrophotometric test (another confirmatory test):** An optical UV-VIS spectrophotometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting UV VIS spectrum is presented as a graph of absorbance (A) versus wavelength.

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the Path length,

**UV calibration method for Rubia dye determination**

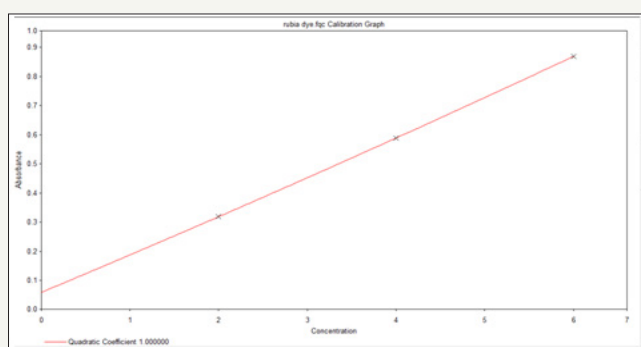


Figure 5: Calibration curve for the rubia dye.

Table 4: Observation from UV VIS Spectrum of natural Rubia and synthetic alizarin.

Peak Reading Received At (Nm)	In Synthetic Red Alizarin	In Natural Rubia	Comment (Describing Difference With Reason)
	250nm (1.38 OD) and 426nm (0.309 OD)	250nm (0.954 OD) and 491nm (0.171 OD)	The Pattern of the Peaks in UV and visible region are very different for the two dye samples

Calibration curve (Figure 5) is done by using 2,4,6,8 and 10mg of Rubia dye per ml of methanol. The solutions need to be filtered through Whatmann filter paper and then used for UV Calibration method and the absorbance shall be measured at 430nm (Table 4).

By several trials, it is observed that the UV-Vis spectrum of aqueous solution of Rubia extract shows peaks at 398nm (0.801)

and 426nm (0.838) as shown in Figure 6 & 7. The said two apparent peaks in this region indicates the presence of natural manjisthin (not synthetic alizarin) clearly visible in enlarged peak of part UV-VIS Spectrum (Figure 7) of Rubiacordifolia extract (used as Natural dye) from Rubia dyed cotton textiles.

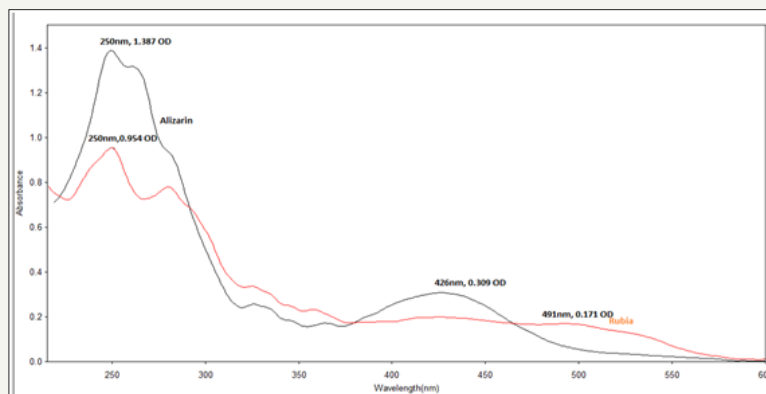


Figure 6: UV spectrum of Rubia and Alizarin dyes.

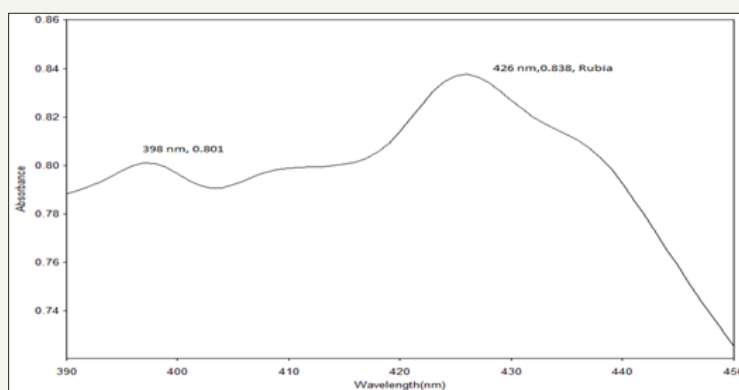


Figure 7: Part of UV-Vis spectrum of natural rubia dye containing manjisthin.

### Conclusion from UV Vis Spectrum Natural Rubia and Synthetic Alizarin

Lambda maxima (optical density) of natural *Rubia* and synthetic alizarin dyes are different. In the UV spectrum, although the peaks are at 250nm but with different optical densities (Figure 6) In the visible region, peaks at 426nm and 491nm for alizarin and peaks at 398nm (0.801) and 426nm (0.838) for Natural Rubia dye containing manjisthin are the characteristic peaks of differentiating alizarin and manjisthin.

Also there are methods developed by FTIR analysis and NMR analysis, which will be available in National Standard Protocol after its all round approval and publication by National and International Standardization body.

However, it is recommended that after preliminary identification by TLC method any two confirmatory tests must be done for certification by any recognised Test laboratory and hence two confirmatory test methods are discussed above.

### Acknowledgement

The authors are grateful to Ministry of Textiles, Govt of India and Office of The Textile Commissioner, Govt. of India, for necessary Project based funding for the R & D Project on "Development of Eco-friendly Natural Fibre Based Textile (OEKO-TECH Products) with simultaneous Natural Dyeing and Natural finishing to establish Natural dye / finish Mark" sanctioned to DJFT,IJT, CU Since Dec-2016 (Sanction no F no. 4(3) /9-B/2016/TDS/143 dated 29-12-2016 and commenced from 01-04-2017.

### References

1. Gulrajani ML, Gupta D (1992) Natural dye and their application to textiles. Department of Textile Technology, Indian Institute of Technology, New Delhi, India.
2. (2002) SERI: Colour from nature silk dyeing using natural dyes, SERI 2000, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, India.
3. Samanta AK, Konar Adwaita (2012) Technical handbook on natural dye and colouration. Department of Jute and Fibre Tech, IJT, Calcutta University, Kolkata, India, pp. 45-72.

4. Bhattacharya N (1999) Natural dye–Its authenticity and identification. Convention Proceedings, 1st Convention on Natural Dyes, Department of Textile Technology, IIT Delhi, India, p. 134.
5. Vankar PS, V Tiwari, Ghorpade B (2001) Supercritical fluid extraction of natural dye from eucalyptus bark. Convention Proceedings, 2nd Convention on Natural Dyes, Department of Textiles Technology, IIT Delhi, India, p. 53.
6. Samanta AK, Agarwal P (2009) Application of natural dyes on textiles. Indian Journal of Fibre & Textile Research 34: 384-399.
7. Tiwari V, Vankar PS (2001) Unconventional natural dyeing using microwave and sonicator with alkanet root bark. Asian Textile J 10(5-6): 54.
8. Samanta AK, Singhee D, Sethia M (2001) Application of single and mixture of selected natural dyes on cotton. Convention Proceedings, 2nd Convention on Natural Dyes, Department of Textiles Technology, IIT Delhi, India, p. 20.
9. Samanta AK, Agarwal P, Konar A, D Siddhartha (2008) Characterization and application of purified selective natural dyes on jute. International Dyer 193(3): 25-33.
10. Samanta AK, Agarwal P (2008) Application of mixture of red sandal wood and other natural dyes for dyeing of jute fabric-studies on dye compatibility. International Dyer 193(3): 37-41.
11. Samanta AK, Agarwal P, Datta Siddhartha (2008) Dyeing of jute with binary mixtures of jackfruit wood and other natural dyes: study on colour performance and dye compatibility. Indian J Fibre & Text Res 33: 171-180.



Creative Commons Attribution 4.0  
International License

For possible submissions Click Here

[Submit Article](#)



### Trends in Textile Engineering & Fashion Technology

#### Benefits of Publishing with us

- High-level peer review and editorial services
- Freely accessible online immediately upon publication
- Authors retain the copyright to their work
- Licensing it under a Creative Commons license
- Visibility through different online platforms