Evaluation of dyeing properties of *Eclipta Alba L.* on protein yarn and their chemical analysis

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Abstract
Dyes are colored substances used to color fibers, leather, paper or foodstuffs etc. Due to the benign nature of dye extracted from natural sources, more importance is given on this area. Natural dyes also protect us from harmful UV rays of the sun and also provide superior quality sensory feelings in contrast to the carcinogenic effect of synthetic dyes.

In this study, natural dyes present in the Eclipta alba L(Hassk) were extracted using conventional techniques under different operating conditions. Potential of the chemicals obtained from the extracts of Eclipta alba. L. was evaluated by coloring pure yarn of silk and wool. Various metal salts were used as mordants to set extracted dye on the fabrics and yarns. The result showed a variation of attractive and earthly green hue on silk and wool. The phyto chemical analysis of the extract reveals that three chemical compounds wedelolactone, apigenin and luteolin are mainly responsible for different shades of bluish green colour extract of the Eclipta alba plant. Spectrochemical analysis was done to identify the compounds present in the dye extract.

Keywords: Mordant, fastness, optimization, isolation, Lab value, K/S value.

Introduction

Colors created by nature are the most striking and attractive aspect in the living world. Colors play an important role in every moment of our lives and strongly influence on the food and beverage we take, clothes we wear, the furnishings in our homes etc. From the pre- historic period, people of India were interested in the art of natural coloring and it was the main ingredient in every aspect of life in each culture. Prior to the advent of Henry Perkin's innovation of organic chemical dye Mauveine in 1856, peoples and artesian of that period extracted most of the dyes from various plant parts including roots, leaves, twigs, stems, heartwood, bark, wood shavings, flowers, fruits, rinds etc.[1]

Obviously, dyes are having complicated chemical composition and co-exist with some chromogen compound like chromophores, auxochrome and some of the other contaminants. The chromophore group is responsible for the color itself where as auxochrome group present in dye compounds has bonding affinity that enhances adherence properties of the dye to the yarn or fabrics. The pigmentary molecules of dye contain aromatic ring structure coupled with a side chain and there is correlation between the chromogen-chromophore structure of compound present in a dye source and its colour yield. The qualitative and quantitative variations of such phyto-constituents depend upon the geographical variations of the plant grown and the different parts of the plant[2].

The hue and intensity of shade are also differed using different mordants in same dye bath. The dye content may vary according to the age, part of the plant, agro climatic conditions and it is important to know the dye content in order to get reproducible shades. Thus, determination of dye content as well as characterization of dye material are important for achieving better performance on natural dyeing processes. It was seen that due to the contaminants co-existing with natural dye source, the identification of actual organic dye compounds is a challenging task. The major barrier of the process is that it is not found in pure form and dye compounds are relatively small in quantity (0.5–5 % only) with low concentration of chromophore group in the compounds.

In recent time, the environmental issue and health benefits specially the UV protection properties of some natural dyes pressurized food as well as the textile industries for use of natural dye on textiles material with more developed functionalities[10]. Recently, number of chemical research in the field of the spectrometric identification of organic dyes have been performed and those studies have presented a new chromatographic technique for the identification of certain dyes compounds for better understanding of dyeing processes[7-9]. As India is one of the few mega biodiversity countries with a rich diversity of flora and fauna, as well as a home for traditional natural dye practices, this study was planned on the same line taking an indigenous herb of Assam, False Daisy (*Eclipta alba* L. Hassk) locally known as Keharaj, distributed in all states of entire North East India.

Material and Methods

Dye plant: The *Eclipta alba(L)* Hassk is one of the easily growing herbs belonging to the family *Asteraceae* and was collected from Sivasagar district of Assam lying between 26.45°N and 27.15°N latitudes and 94.25°E and 95.25°E longitudes of Assam, India.

The plants were washed thoroughly with water to remove its dirt and dust. They were air dried and grinded into very small units with the help of a grinding machine. It is noted that

powdered and dried materials can be stored in airtight bags and containers for at least a year and can be used for dyeing whenever required.

Selection of substrates: The yarn selected for the study was the high fashion luxurious mulberry silk of Assam, which has better affinity for dye along with its good penetrability. Secondly, the pure wool yarn was procured from Ludhiana, Punjab, India.

Selection of mordants: Most of the natural dyes need a substance- metal salt as color intensifier called mordant. They are those metals which form a prirnary complex on one side with the yarn and on the other side with the dye and thus help in chemical bonding between dye and fibers of the yarn. Bio-mordant mybrobalan (*TerminiliaChebula*) seed and eco-friendly mordant alum ($AlKSO_2$) were applied in dyeing of silk and wool yarn.

Selection of chemicals and reagents: Reagents used for the study were sodium carbonate (Na_2CO_3), ethanol (C_2H_5OH), methanol (CH_3OH), dichlromethane (CH_2Cl_2), chloroform ($CHCl_3$) and acetone (CH_3COCH_3) for experimental work. The chromatographic material silica gel (Chromatographic grade 100-200 mesh for column), solvent like hexane (C_6H_{14}), ethyl acetate ($C_4H_8O_2$) were extensively used for the study.

All the solvents were distilled before use. Soft and pure water were used for dye extraction, preparation of mordent solution for dyeing and washing procedure.

Methods

Preparation of yarn for dyeing: The mulberry silk yarn having highest sericin content (21%), degummed with mild alkali solution and already degummed and bleached wool were used for the study.

Preparation of dye powder: The air dried plants were grinded with an electrical grinder to make fine particle as the smaller particles give better results in dyeing of yarn and decreased particle size shortens the time required for dyeing. For proper dye extract, the size of dye particles was kept in a standard size i.e. in between 150-300 microns or 50-100 mesh through sieve analyzer and bigger particles are disposed off[12].

Optimization of dyeing conditions: A series of experiments were conducted to optimize the different dyeing conditions namely material to liquor ratio for extraction, dye extraction medium, alkali or alcohol concentration for extraction of dye, time and temperature of dye extraction. Dye material concentration, dyeing time, concentration of mordants, mordanting time, mordanting methods for dyeing of silk and wool yarns are also important.

Alkaline extraction: Respective dye powder was taken and poured in boiling water and 5% Na_2CO_3 was added. The water bath was kept at 60 ° C for about one hour so as to extract all the colour from them[3]. The extract was cooled down and finally filtered with fine filter paper for three times to ensure clear dye solution. The optical density (OD) value was measured by using UV visible spectrophotometer. The dye extracts obtained at different pH values were used for obtaining standard calibration curves through their absorbance values found using a dual beam reflectance spectrophotometer.

Alcoholic extraction: The dye powder was subjected to Soxhlet extraction, using methanol as a solvent. The cycle is repeated for three times at 60 ºC. Then the cooled extract was filtered through a filter paper and solvent was removed through a rotary evaporator.

pH of the dye: Majority of the dyes are anionic in nature and have strong coordination to make best interaction with cationic substance. To ascertain the nature of the dye, the pH of the dye solution was measured before carrying out the dyeing process. It was also noted that alkaline pH should be avoided for dyeing of protein fibers[4,5]. The absorbance value of extracted dye liquor at the pH 7.0 was considered as standard[19].

Dyeing of silk and wool yarns: After optimization of different parameters such as dye powders, time and temperature, the wet silk and wool samples were dyed as per optimized condition in a dye bath. To obtain different colour shades, simultaneously mordanting was done with two different eco-friendly mordants myrobalan and alum. The movement of textile material in the dye bath is very essential. The yarns need to be continuously stirred in the dye bath, otherwise uneven dyeing may result.

After the dyeing was over, the dyed materials were removed and allowed to cool down a little and then washed with water. The washed dyed material was then treated with a hot soap or non-ionic detergent solution to remove loosely held dye and again rinsed with water and air dried in shade at room temperature to evaluate the ability of dye to withstand various agencies. The colorfastness, strength(K/S) and colour coordinator in term of CIE Lab value were evaluated[6]. Shade cards for colour catalogue were prepared using the dyed yarns.

Colour Fastness properties: The colour fastness grade of dye on silk and wool yarns with mordants against washing and sunlight were tested by standard test methods (ASTM Standard,1968). The effect of color on the test specimens was expressed in the international grey scale grade by grading the sample as:

1-Very poor, 2-poor, 3-fair, 4 –very fair, 5-good and 6-very good

Colour strength (K/S): The other qualities like colour strength (K/S) and CIE lab values of dyed samples were

determined by light reflected techniques using UV/VIS spectrophotometer. K/S values were determined using Kubelka Monk equation mentioned as:

$K/S=[(1-R)^2/2R]$

where R is observe reflectance, K is the absorption co-efficient and S is the light scattering co-efficient[17].

CIE Lab values of dye: The dyeing results or colour coordinates of the dyed samples were determined based on the CIE lab system via the spectrophotometer[6].

Phyto-chemical analysis: For isolation of colour compounds present in the dye, the greenish colour extract of dye compound was initially tested by TLC plates. Then the sample was injected in the chromatographic column packed by silica gel eluted with mixture of ethyl acetate and hexane and different pure compounds were isolated. The isolated colour compounds from the dye particles were identified by using the analytical studies: IR in Perkin Elmer spectrophotometer (Spectrum -100), Ultra violet (U/S) Spectroscopy, ^{I}H NMR and C^{13} NMR: Nuclear Magnetic Resonance (Bruker AV-DPX-300 and 500 MHz) in $CDCl_3$, MS Spectra in GC-MS spectrophotometer and element analysis through Perkin Elmer series II CHN PE- 2400 element analyzer.

Results and Discussion

Size of the dye particle: The highest weight percentage of grinded dye powder used for the study was 43.35 % of standard size 150-300 µm followed by 33 % of a little bigger size 300-500 µm (Fig.1).

Optimum dyeing condition: The dye solution, which gave the maximum colour strength, was utilized to optimize the extraction levels of material-to-liquor ratio, temperature and time. The optimum dyeing conditions for extraction of best quality colour for dyeing of natural yarn were found as follow in table 1.

Mordants and methods of modanting: The use of mordant produced different shades on silk and wool yarn. Moreover, it was seen that application of mordant increased the color yield. It was because of one molecule of dye can form a bond with one site of fiber molecule while one molecule of mordant can form bonds with two or more molecules of dyes[5]. Myrobalan mordanted dyed samples appeared in dull colour due to the presence of tannin which invariably reduced the chroma of shade[13]. Marked change of color was observed in different lighting conditions highly supported by Shivankar et al as it was stated that in a single plant, one may obtain between 5-15 varying color shades[21].

Dyed sample: In the dyed yarns samples, it was noticed that the colour shades produced by the whole plant of *Eclipta alba* L. were with variation of bluish greenish tint (Table 2). It was obvious that the creation of specific colours on yarn samples depended on some interaction factors such as pH and chemical interactions of dye compounds with substrates. During dyeing of protein fibres in acidic condition, the amino group of silk became positively charged and attracts the negatively charged dye anion[11].

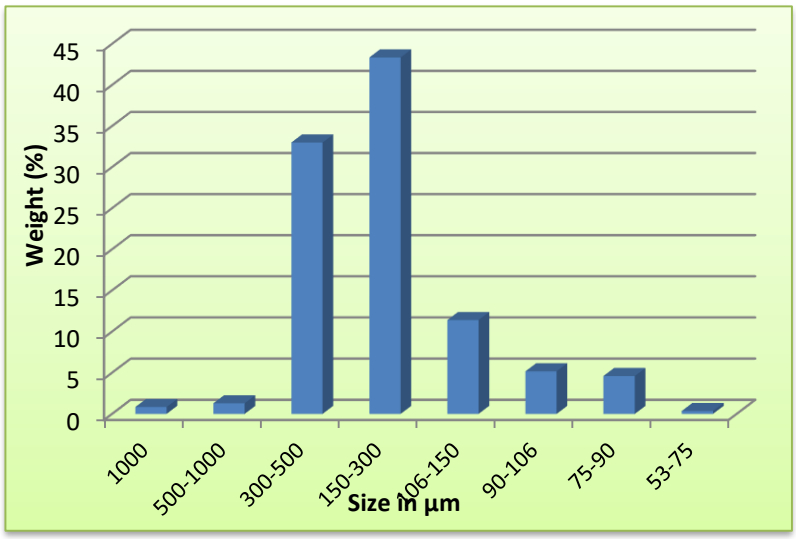


Fig. 1: Particle size distribution of dye

Table 1
Optimized condition for dyeing of yarns (silk, wool)

pH of dye liquors	Dyeing parameters				
	Dye conc. gm/100ml	M:L ratio	Dyeing temp. (°C)	Dyeing time (min)	Mordant (%)
7.8	5	1:40	70-10	45	0.8-1.0

Fastness properties: The color fastness properties of the dye were found to be good with both the mordants. The co-exist of chromophores auxochrome groups of the dye, rate of the diffusion of the dye and the dye site of fiber are mainly responsible for colorfastness (wash and light fastness) of both the silk and wool yarn[16]. Table 3 showed the fastness properties of dyed yarns.

The colour strength (K/S) value of a dye is one of the key values in reflecting the surface lustre of the dyed products. The K/S values of yarns dyed with myrobalan and alum mordants were presented in the table 4 along with the CIE Lab values in terms of redness, blueness, greenness and yellowness of dye. Table 4 indicated that among the color strength values of silk and wool, the wool yarns showed highest K/S values with both the mordants. This is due to the hygroscopic nature of wool yarn with many amorphous regions in its molecular structure[21] which led to the swelling of fiber, hence the color strength of wool yarn was higher than silk[14,22].

Wool molecules are present in a flexible chain and bind together with a natural cross linkage and salt bridge that helped the attraction of more dye molecules to the vicinity of the fibers and increased dye ability[20].

Phyto-chemical analysis for identification of chemical structure of *Eclipta alba* dye: The result of analytical studies such as IR, UV, ^{1}H NMR, ^{13}C NMR and mass spectroscopy and melting point analysis identified the colour compounds present on the plant part.

Table 2
Dyed silk and wool yarns

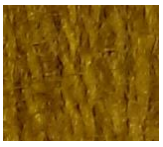
Name of yarns	Mordants	
	Alum	Myrobalam
Silk		
Wool		

Table 3
Rating for wash and light fastness properties

Mordant used	Wash fastness				Light fastness			
	Silk		Wool		Silk		Wool	
	CC	CS	CC	CS	CC	CS	CC	CS
Myrobalan	4	5	4	5	4	5	4	5
Alum	4	5	4	5	3	5	4	5

CC: Color change CS: Color staining
CC Ratings: 1=Very poor, 2=poor, 3=fair, 4=good, 5= very good,
CS Ratting: 1= heavily stained, 2=considerably stained, 3 = noticeable stained, 4 = slightly stained, 5= negligible or no staining.

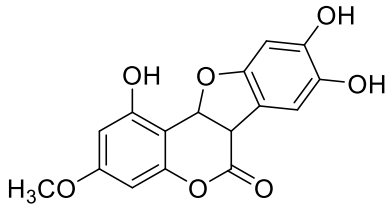
Table 4
TheColor strength (K/S) and CIE Lab values of the dye

S.N.	Mordants	K/S	CIE Lab Values		
			L*	a*	b*
Silk	Myrobalan	6.22	26.89	-27.73	61.12
	Alum	5.17	43.75	-7.94	-66.15
Wool	Myrobalan	8.72	46.31	-44.78	5.75
	Alum	5.66	51.54	47.52	12.34

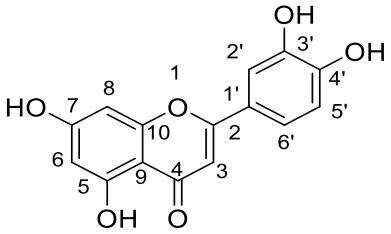
L*= depth of color (lower value of L* indicate higher depth of color).
a*= redness (positive a*) and greenness (negative a*)
b*= yellowness (positive b*) and blueness (negative b*)

Characterization of colour compounds

Compound E-1: Yield: 0.20 mg (yellow needle), R*f* value 0.68, mp 300 °C. UV λ_{max}: 224 nm, IR (KBr pellet) ν_{max}: 1727.0 cm^{-1}, ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.23 (s, 1H), 7.15 (s, 1H), 6.55 (d, *J*= 1.9 Hz, 1H), 6.40 (d, *J*= 1.8 Hz, 1H), 3.22 (s, 3H) ppm, ^{13}C NMR (500 MHz DMSO-d_6,): δ 162.2, 158.9, 157.8, 155.3, 154.8, 148.9, 145.4, 144.3, 113.6, 104.5, 101.7, 98.8, 98.1, 96.7, 93.2, 55.7 ppm, MS(ESI) (m/z): 314 (M^+). Compound E-I is identified as wedelolactone. *Eclipta alba L*. has a wide range of chemical compounds and the leaves contains 1.6% wedelolactone[8].

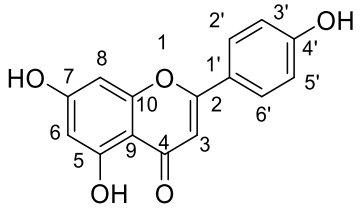


Compound E-II: Yield (yellow needle): 0.24 mg, R*f* value 0.347, Melting point: 327-328°C, UV $\lambda_{max,}$ 254 nm, IR ν_{max}($CDCl_3$) 3424, 1638, 1520 cm^{-1}, ^{1} H NMR (500 MHz $CDCL_3$) δ12.95(s, 1H), 10.86(s, 1H), 9.95(s, 1H), 9.44(s, 1H), 7.42 (m, 2H), 6.9 (d, *J*= 8Hz, 1H), 6.6 (s. 1H), 6.4 (d, *J*=1.5Hz, IH), 6.1(d, *J*=1.5Hz, IH) ppm,^{13}C NMR(500MHzCDCl$_3$), δ 182.2, 164.2, 161.7, 157.6 ,150.0 , 146.0, 121.7, 119.3, 116.3, 113.6, 104.0, 103.1, 99.0, 94.1 ppm, (m/z) 286[M^+], Analysis for $C_{15}H_{10}O_6$, Calculated : C 62.93%, H 3.49%, Found C 62.94% H:3.51 %. Compound E-II is identified as luteolin.



Structure of Luteolin

Compound E-III: Yield (yellow needle): 0.28 mg, R*f* value 0.6, Melting point: 432°C, UV $\lambda_{max,}$ 268,331 nm, IR ν_{max}(KBr pellet) 332.7 1651,1608,1244,1181cm^{-1}, ^{1} H NMR (500 MHz $CDCL_3$) δ 12.9 (s, 1H), 10.6 (s, 1H), 10.54 (s, 1H), 7.9 (d,*J*=8.5Hz, 2H), 6.9 (d, *J*= 8.5Hz, 2H), 6.7(s. 1H), 6.1(s, IH), 5.8 (s,1H) ppm,^{13}C NMR(500MHz, $CDCl_3$), δ 182.0, 164.4, 164.0, 161.7., 161.4, 157.6 , 128.7, 121.5, 116.2, 104.0, 103.7, 99.1, 94.3. ppm, MS(ESI) m/z: 270[M^+], Compound E-III is identified as apigenin.



Structure of Apigenin.

Scientifically, it was proved that the main color yielding component of the plant is wedelolactone responsible for green colour whereas luteolin and apigenin are the basic cause of its blue tint though it is not reported in any literature[15]. Those compounds present in the plant help in fighting against hepatitis and snake venoms and further examination of the constituents of this plant is currently in progress. Beside silk and wool yarn, other fiber can be dyed using these colour compounds and can get a variation of attractive and earthly green hue with other natural mordants. Thus, the herb proved to be a most valuable source of natural color for diversified textiles products requiring coloration.

Conclusion

To move with the ecological movement in the earth with sustainable products, the natural dyes and their chemical investigation of dye compounds present in dyes source (plant and animal) are highly acknowledged in dyeing processes. In the field of textiles, characterization of chemical nature of colored components is useful to know the solubility, mordanting power of natural dye as well as hypo-chromic and batho-chromic shift of main hue.

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