



# *Natural Dyes: Application, Identification and Standardization*

**By:**

**Dr. Ruma Chakrabarti  
and**

**A. Vignesh**

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## **ABSTRACT**

Natural dyes comprises of those colourants (dyes and pigments) that are obtained from animal or vegetable matter without chemical processing. They are mainly mordant dyes although some vat, solvent, pigment, and acid types are known.

Natural dyes find use in the colouration of textiles, foods, drugs, and cosmetics. Small quantities of dyes are also used in colouration of paper, leather, shoe polish, wood, cane, candles, etc. In the earlier days, dyes were derived only from natural sources. But natural dyes suffer from certain inherent disadvantages of standardized application and the standardization of the dye itself as dyes collected from similar plants or natural sources are influenced and subjected to the vagaries of climate, soil, cultivation methods etc. Hence for the natural dyes to be truly commercialised and to take a competitive place with respect to the synthetic dyes, the standardization methods play a very significant and vital role. In this paper we shall indicate some conventional and often used methods of application, the identification methods and also standardization process of the natural dyes.

**Natural dyes** comprises of those colourants (dyes and pigments) that are obtained from animal or vegetable matter without chemical processing. They are mainly mordant dyes although some vat, solvent, pigment, and acid types are known.

Natural dyes find use in the colouration of textiles, foods, drugs, and cosmetics. Small quantities of dyes are also used in colouration of paper, leather, shoe polish, wood, cane, candles, etc. In the earlier days, dyes were derived only from natural sources. Some processing was required but essentially the dye itself was obtained from a plant, mineral or animal. After the accidental synthesis of mauveine by William Henry Perkin in 1856 and its subsequent commercialization, heralding the advent of coal tar dyes (now synthetic dyes), the use of natural dyes receded. A wide

range of synthetic dyestuff was thrust upon the industry and it was readily accepted for its distinct advantage over natural dyes with respect to application, colour range and availability.

**Status of Natural Dyes in India:** India has a very rich tradition of using natural dyes. The art and craft of producing natural dyed textile has been practiced since ages in many villages by traditional expert crafts-persons in the country. Natural dyes, when used by themselves have many limitations of fastness and brilliancy of shade. However, when used along with metallic mordants they produce bright and fast colours. The use of metallic mordants is not always eco-friendly, but the pollution problems created by metallic mordants are of very low order and can be easily overcome. Therefore, instead of using unsustainable technology for producing colours one can use 'Mild Chemistry' to achieve almost similar results.

In the past decade the synthetic dye manufacturing has come under the tight scrutiny of the public eye due to the following reasons: -

**a)** Certain chemical compounds used in chemical dyes are found to be carcinogenic, mutagenic and sensitizing and as a result, they have been banned. Azo dyes have been red listed. Therefore manufactures of handloom goods and those promoting their exports have come to realize that the developed countries like the US, Germany, Austria and Denmark patronizing Indian handloom prefer garments coloured with natural dyes as compared to synthetic dyes.

This in itself provided the main thrust for the retrospection by the dyeing industry and the revival of interest in natural dyes and the introduction of eco-friendly natural dyes as an alternate to the existing synthetic dyes.

**b)** There is a growing awareness and public concern in recent years over the issue environmental pollution and hazards arising out of the textile dyeing industry.

Dyeing of fabrics with natural dyes, a rich traditional craft is now therefore being revived.

In recent years an interest in natural dyes has been manifested as,

- Reconstruction of ancient and traditional dyeing technology.
- Study of Museum textiles and textiles recovered by Archaeology.
- Conservation and restoration of old textiles.

- Growing use of natural dyes in home craft works.
- Chemical characterization of colourant in flora and fauna.
- Replacement of synthetic dyes by natural dyes for foods, safety and so forth.<sup>2,3,4.</sup>

### **Stake Holders of Natural Dyes: -**

The use of natural dyes has increased substantially during the last couple of years. These dyes are being mainly used by<sup>3</sup>:

- ✓ Hobby groups
- ✓ Designers
- ✓ Traditional dyers and printers
- ✓ Non-government organizations (NGO's)
- ✓ Museums
- ✓ Academic institute and research associations/laboratories
- ✓ Industry.

## **CLASSIFICATION of NATURAL DYES**

Mainly three types of natural dye classification are done based on Chemical Class, Application Class and on Colour.

### **1. BASED on CHEMICAL CLASSES: -**

The natural organic dyes and pigments cover a wide range of chemical classes. viz. Polymethines, Ketones, Imines, Quinines, Anthraquinonoids, Naphthoquinones, Flavones, Flavanols, Flavanones, Indigoids and Chlorophyll. Some of the important chemical classes are enumerated below.

### **BASED ON APPLICATION CLASS: -**

Bancroft, in his, "Treatise on Permanent Colours" published about 160 years ago, classified natural dyes into two groups.

- **Substantive dyes:** -The dyes, which dye the fibre directly, are classified as substantive dyes.

Example: Indigo, Turmeric, Orchil.

- **Adjective dyes:** - These dyes dye the material only when it is mordanted with metallic salt or with addition of metallic salt to the dye bath.

Example: Logwood, Madder, Cochineal, Fustic.

In pure state, the adjective dyes are only slightly coloured and when used alone give poor dyeing.

The above classification was replaced by an equivalent subsequent classification as **Direct dyes and Mordant dyes**.

Another classification from **Hummel** is as follows

- **Monogenetic dyes:** - These dyes produce only one colour irrespective of the mordant.
- **Polygenetic dyes:** - The colour generated by these dyes depends on mordant used.

Another classification based on application<sup>4</sup>:-

### **2.2.1. Mordant Dyes:** -

Mordant dyes are defined as those dyes, which have affinity for mordanted fibres. All dyes, which form complex with mordants, are grouped under mordant dyes.

Example: Fustic, Kermes, Cochineal etc.

Many natural dyes are extracted along with tannin from the vegetable matter and the dye is directly absorbed by untreated cotton fibres. In such case, mordanting and dyeing takes place simultaneously. Tannin, being brownish in colour, has a tendency to modify the hue, making dull.

In some cases, cotton fabric is treated with tannin or tannin containing vegetable matter such as harda and subsequently mordanted with metallic mordant. In this case, the tannin holds the metal and the metal forms complex with dye.

### **2.2.2. Vat Dyes:** -

The 'Vat Dye' derived their name from the wooden fermentation vessel called 'Vat', which was at one time used for reducing the dye in order to convert it into soluble form. This process of solubilization is called Vatting and the soluble form of dye is called leuco dye. The soluble leuco form has affinity for natural fibres, which can be oxidized back on fibre on exposure to air. The



vatting is also carried out by treatment with reducing agent, such as sodium hydrosulphite and alkali, such as sodium hydroxide. Example: Indigo

### **2.2.3. Direct Dyes: -**

e.g. Turmeric, which though a fugitive dye has an affinity for cotton. This dye is also directly sorbed by other natural fibres.

Examples: Turmeric, Annatto, Harda, pomegranate (*Punica granatum* L.), Carthamin obtained from Safflower etc.

### **2.2.4. Acid Dyes: -**

Acid dyes are type of direct dyes for polyamide fibres like wool and silk. These dyes are applied from acidic medium and they have either sulphonic or carboxylic group(s) in the molecule. Example: Saffron.

An aftertreatment with tannic acid and tartar emetic, known as back tanning, improves the wash fastness of these types of dyes.

### **2.2.5. Basic Dyes: -**

These are cationic dyes, which on ionization gives coloured cations. They are used on polyamide fibres such as wool and silk from neutral or mildly acidic conditions. These dyes may be applied on cotton mordanted with tannic acid and tartar emetic or other metal salts. These dyes have low light fastness.

Example: Barberine.

### **2.2.6. Disperse Dyes: -**

A disperse dye has a low relative molecular mass (r.m.m.), low solubility and no strong solubilising groups. These dyes have hydroxyl and/ or amino groups which imparts some solubility to the dye molecule. One such dye could be Lawsone. Many other flavone and Anthraquinone dyes can qualify to be classified as Disperse dyes.

### **2.3. BASED ON COLOUR: -**

In the Colour Index, the dyes are classified according to chemical constitution as well as major application classes. Within application class, the dyes are arranged according to hue. Natural dyes form a separate section. The no of dyes in each hue are given below. Some dyes produce more than one hue.

| <b>C.I. NATURAL</b> | <b>NO. OF DYES</b> | <b>PERCENT</b> |
|---------------------|--------------------|----------------|
| YELLOW              | 28                 | 30.4           |
| ORANGE              | 6                  | 6.5            |
| RED                 | 32                 | 34.8           |
| BLUE                | 3                  | 3.3            |
| GREEN               | 5                  | 5.5            |
| BROWN               | 12                 | 13.0           |
| BLACK               | 6                  | 6.5            |

Of the 92 natural dyes listed in Colour Index, chemical structure of 67 dyes is disclosed. Many dyes have more than one compound and some dyes have identical structures.<sup>4</sup>

#### **Some Important Natural Dyes: -**

Colouring matter could be obtained from all almost all vegetable matter. However, only a few of these sources yield colourants which can be extracted and work out to be commercially viable. Similar is the case of colourants obtained from animal origin.

Basically, three primary colours are required to get any given hue (or colour). This type of approach has been worked out for synthetic dyes. However, in the case of natural dyes, the dyeing procedures are different for different dyes and they cannot be blended to get the required colour easily. Never-the-less, while looking for different colours, it is better to have a limited number of dyes with good fastness properties rather than having too many colours (sources) with limited fastness properties.

While selecting the proper palette of colours, one would like to have atleast one blue, one red and a yellow to start with. Due to the limited number of natural dyes available, the correct choice of the dyes is very important. Information on some important natural dyes is given below.<sup>3</sup>

**Blue Dyes:** - The Colour Index lists only three natural blue dyes; namely Natural Indigo, sulphonated natural Indigo and the flowers of the Japanese ‘Tsuyukusa’ used mainly for making awobana paper. The only viable choice among the blue natural dyes is Indigo. **Natural indigo** is obtained by fermenting the leaves of various species of Indigofera, running off the liquor and oxidizing it to precipitate the dye. **Woad** (*Isatis Tinctoria L.*) is another source of indigo. The main ingredients of natural indigo are Indigotin and Indirubin. Natural indigo has higher affinity and the dyed fabrics have better fastness.

**Red Dyes:** - The Colour Index lists 32 red natural dyes. The prominent among them are **Madder** (*Rubia tinctorum L.*), **Manjeet** (*Rubia cordifolia L.*), **Brazilwood/Sappan wood** (*Caesalpinia sappan L.*), **Al or Morinda** (*Morinda citrifolia L.*), **Cochineal** (*Coccus cactil L.*) and **Lac dye** (*Coccus laccae*). All these dyes are based on anthraquinone molecule except Brazil and Sappan wood based dyes. These dyes are prone to oxidation and hence are not suitable.

The strong and almost fadeless cotton dye known as **Turkey Red** was developed in India and spread from there to Turkey. It involved about twenty separate processes using wood, oil and rancid fat, charcoal, cow/sheep/dog dung, and liquid content of animal stomach. Only the dyers and their families not surprisingly, occupied villages where the process was carried out. The use of madder declined abruptly following the development of synthetic alizarin in 1869.

**Yellow Dyes:** - Yellow is the most common colour in the natural dyes. However, most of the yellow colourants are fugitive. The Colour Index lists 28 yellow dyes. Some of the important yellow dyes are obtained from **Barberry** (*Berberis aristata*), **Tesu flowers** (*Butea frondosa*, *Monosperma*) and **Kamala** (*Mallotus philippensis*).

Other sources of yellow dyes are **Black oak** (*Quercus velutina*), **Turmeric** (*Curcuma longa*), **Weld** (*Reseda luteola*) and **Himalayan rhubarb** (*Rheum emodi*).



## **MORDANTS: -**

Mordant is a chemical, which can fix itself on the fibre and combines with the dyestuff. A link is therefore formed between the dyestuff and the fibre, which allows certain dyes with no affinity to be fixed on to the fibre.

**Types of Mordants:** - Three types of mordants namely,

### **Metal Salts or Metallic Mordants.**

Several different metal salts can be used for mordanting. The six most effective ones are

- Alum – Potassium aluminum sulphate
- Copper – Copper sulphate
- Chrome – Potassium dichromate
- Iron – Ferrous sulphate
- Tin – Stannous chloride, Stannic chloride.

### **Tannins and Tannic acid.**

Tannins are naturally occurring compounds high mol. wt (500-3000) containing phenolic hydroxyl groups (1-2 per 100 Mw) to enable them to form effective crosslinks between proteins and other macromolecules. The vegetable tannins divided structurally into two distinct classes depending on the type of phenolic nuclei involved and the way they are joined together.

➤ Hydrolysable tannins obtained from myrobalan fruit, oak bark, gallnuts,

Pomegranate rind, sumac leaves.

➤ Condensed tannins like catechin obtained from acacia catechu.

Tannins such as harda, tannic acid, etc are considered as natural mordants.

Gallnut contains 60-77% of Tannic Acid.

### **Oil or Oil Mordants: -**

Oil –mordants are mainly used in the dyeing of Turkey Red Colour from madder. The main function of the oil-mordant is to form a complex with alum used as the main mordant. Since alum is soluble in water and does not have affinity for cotton, it is easily washed out from the treated fabric. The naturally occurring oils contain fatty acids such as palmitic, stearic, oleic, ricinolic etc. and their glycerides. The –COOH groups of fatty acids react with metal salts and get converted

into  $-\text{COOM}$ , where M denotes the metal, for instance in the case of alum it would be Al. subsequently, it was found that the treatment of oils with concentrated sulphuric acid produces sulphonated oils which possess better metal binding capacity than the natural oils due to the introduction of sulphonic acid group,  $-\text{SO}_3\text{H}$ . The sulphonic acid can react with metal salts to produce  $-\text{SO}_3\text{M}$ . The bound metal can then form a complex with the mordant dye such as madder to give Turkey Red colour of superior fastness and hue.

### **Techniques of Mordanting: -**

A subsequent treatment of the tannin treated substrate with metal salts such as alum introduces aluminum ions in the fibre. The tannin treated material at the hydroxyl or carboxyl groups sorbs these ions, either by forming a metal-complex or metal salt. These metal ions then provide sites for mordant dyes. Hence introducing metal-ions in the fibre. Either directly or as tannin-metal complexes can increase the affinity of the substrates towards natural dyes. Therefore, tannins by themselves do not act as mordants but the tannin-metal salt combination acts as a mordant for the natural mordant dyes.

### **Methods of Mordanting: -**

The three methods used for mordanting are: -

- Pre-mordanting: - The substrate is treated with the mordant and then dyed.
- Meta-mordanting: - The mordant is added in the dyebath itself.
- Post-mordanting: - The dyed material is treated with a mordant.

The methods have different effects on the shade obtained after dyeing and also on the fastness properties.

It also depends upon the dye and the substrate. It is therefore necessary to choose a proper method to get the required shade and fastness by optimization of parameters. <sup>3,4</sup>.

## **NATURAL DYES-“TECHNIQUES OF EXTRACTIONS”:-**

Natural Dyes cannot be used directly from their renewable sources. Using raw materials for dyeing has many limitations. Safe and cheap extraction of main colouring component is most important without affecting the extraction conditions and avoiding any contamination in various extraction techniques. Several Extraction Methodologies for natural dye that comply with both consumer preference and regulatory control and that are cost effective are becoming more popular. Techniques of Extractions of Natural Dyes<sup>5</sup> involve

- ‘Simple Aqueous Methods’
- ‘Complicated Solvent Systems’
- ‘Ultrasonic Extraction in Sonicator’
- ‘Supercritical Fluid Extraction Techniques’.

### **Simple Aqueous Methods:-**

Natural dyes are mixed with required amount of water and boiling for optimum time (found out by optimization of parameters) which is 60 minutes in most cases. The content is cooled to Room Temperature and filtered. The filtrate is used as a dye for dyeing.

Example: -Extraction of **Onion** Dye: - the outermost dry papery skins of onion were removed and boiled with water for 1 hour. The content was cooled to a R.T. and filtered. The filtrate was used as the dye solution.<sup>9</sup>

### **Complicated Solvent Systems:-**

The dried material (leaves, roots, barks, wood, resinous secretion of insects etc) are ground to very fine particles. The crude dried powder is weighed and solvent extracted using Soxhlet Apparatus, Steam Heated Extractor. Different solvents (such as Acetone, chloroform, ether, n-hexane, alcohol, soda ash, etc.) are used for Extraction. The process is carried out for 4 hours. The dye extract is evaporated in an evaporating dish over a water bath. After evaporating to dryness, the solute is weighed and the percentage yield is calculated.

Example: - Extraction of **Henna** (lele) using solvent: - The dried leaves were ground to very fine particles. 40 gms of the crude dried powder was weighed and solvent extracted using a Soxhlet apparatus. Three different solvents acetone, chloroform and water were used for the extraction. 250 ml of each solvent was used. The process was carried out for four hrs.

The dye extract was evaporated in an evaporating dish over a water bath. After evaporating to dryness, the solute was weighed and the percentage yield is calculated.<sup>10</sup>

### **Ultrasonic Extraction in Sonicator: -**

The combined effect associated with Ultra-Sound Energy is of cavitations, compressions, rarefactions, and microstreaming results in intermolecular tearing and surface scrubbing. In particular, it has been noted that, some reactions when exposed to ultrasonic energy become faster with lower temperature that is the most beneficial effect as it reduces processing time and energy consumption and improves product quality in the colouration of textiles.

Example: -**Sappanwood** extract is obtained from boiling the matured wood in hot water from the chips or rasped wood and extraction is preferably done on counter current principle, but even boiling under pressure gives stronger decoction. However, this procedure has serious defects heat changes the colour of red dye to brown. To overcome this drawback we use Sonicator for extraction of dye in water using ultra-sound energy. Optical Density is recorded to evaluate the relative dye content. The extraction of the dye by usual technique required long hours of immersion of the wood chipping in water or heating. Both these processes are causing the dye to decompose due to oxidation. To extract the dye faster and in a more efficient manner Sonicator with Ultra-Sound Energy of 20 kHz frequency is used. Due to its fugitive character, Sappanwood has found little use, only some cottage dyers in some remote villages found use of this where synthetic dyestuff was not available.

Ultrasonic Extraction Followed by **Microwave Extraction** for solids found extensive use but mainly based on organic solvent extraction.<sup>6,7,8.</sup>

### **Supercritical Fluid Extraction Techniques: -**

No doubt, safety of both producers and consumers is now a major requirement of any new product or process. Accordingly, compelling regulations on the usage of hazardous, carcinogenic, or toxic solvents as well as high energy costs for solvent regeneration have curtailed the growth of the natural extract industries. To suppress the competitive edge of synthetic materials, alternative

extraction methodologies are used. One of such major technologies that have emerged over the last two decades as the alternative to the traditional solvent extraction of natural products is the Supercritical Fluid Extraction Techniques. It uses a clean, safe, inexpensive, nonflammable, nontoxic, environment-friendly, non-polluting solvent, such as CO<sub>2</sub>.

Besides the energy cost associated with this Novel Extraction Technique are lower than the costs for traditional solvent extraction method. Supercritical Fluid Extraction Technology is thus increasingly gaining importance over the conventional techniques for extraction of natural products.

A large body of experimental data has been accumulated on the solubility and extractability of natural products, such as steroids, alkaloids, anticancer agent, oils from seeds, and caffeine from coffee beans, in various SCF solvent such as CO<sub>2</sub>, ethane, ethylene, and N<sub>2</sub>O. CO<sub>2</sub> is the most widely investigated SCF solvent since its Critical Temperature ( $T_c = 31.1^\circ\text{C}$ ) makes it an ideal solvent for extracting materials that are thermally liable.

Supercritical fluid extraction is an advanced separation technique based on the enhanced solvating power of gases above their critical point. CO<sub>2</sub> is an ideal solvent in the food, dye, pharmaceutical and cosmetic industries, where it is essential to obtain final products of a high degree of purity.

Example: - Supercritical CO<sub>2</sub> Extraction of **lycopene** and **β-carotene** from **ripe tomatoes**. Main pigments of ripe tomatoes are the carotenes, compounds of colours ranging between yellow and red, i.e. α, β and γ-carotene, lycopene and xanthophylls at very low concentrations. Experimental: -Supercritical CO<sub>2</sub> extraction are performed by an SFE-400 thermal pump (Supelco), equipped with a 10 cm<sup>3</sup> internal volume extractor. The extraction pressure could be adjusted in 100 psi increment upto a maximum pressure of 4000 psi. The temperature range is 30-200 °C. The extracted substances are recovered in a vial connected to a restrictor. At each run, the rate flow is maintained at about 500 cm<sup>3</sup>/min. Several methods are used for the total or partial removal of water from the tomato samples submitted to the supercritical fluid. In all cases, the samples are protected from the action of both light and oxygen in the air in order to prevent them from damaging the dye. The extractions are performed on ripe tomato pulp or dry skins, since they contain about 5 times as much lycopene as in the whole tomato pulp.

The extractions are initially conducted on completely fresh tomatoes, ground and filtered in a vacuum. In order to adsorb the remaining moisture, the samples are mixed with silica gel. The measurements are carried out on 'camone' greenhouse tomatoes, grown in Sardinia and on field

tomatoes. Extractions are subsequently carried out on the skins and dried seeds of field tomatoes. The tomato skins and seeds are dried for 24 hours in an air drier at 35 °C and stored at -5 °C, the product is ground before being extracted. The extractions are run by submitting 2.5gm of fresh or dry ground sample to different pressures (2500-4000 psi) and temperatures (40-80 °C) for 30 min. both in the presence (1ml) and absence of an entrainer. The extract coming out of the restrictor are collected in ethanol and analyzed by **HPLC**.<sup>11, 12.</sup>

### **IDENTIFICATION of NATURAL DYES: -**

Today natural dyes are used in limited quantities by craftsman in various parts of the world. Although difficult to obtain commercially, dyes are readily obtained from plant sources viz., flowers, leaves, barks, seeds, roots, etc. Craftsman are becoming gradually enthusiastic about this out-dated and time consuming process for one of the reasons that natural dyestuffs procedure offbeat, one-of-a kind colour. There are not two lots identical, each having subtle differences due to impurities peculiar to the particular plant material used. Thus, the very characteristics of natural dyes that made the early dyers despair, appeal to today's craftsman searching for the unique.

Since last decade application of natural dyes on cotton, silk, wool, jute is gaining popularity all over the world possibly due to the context of German ban on synthetic Azo dyes which are based on carcinogenic or allergic arylamines. Natural dyes derived from flora and fauna are believed to be safe but sometimes the method of application using metallic mordants makes them non-ecofriendly to humans and environment. However, natural dyes by virtue of their unique colour/shade and aesthetic characteristics have a potential export market. Recently, the buyers from EEC countries especially from Germany insist on a certificate for eco-friendliness of the commodities and therefore, testing of natural dyed substrates as per Eco-standard as well as their authenticity has become imperative.

Literature survey reveals that some work has been reported an extraction and identification of natural dyes from historic and archeological textiles but a systematic approach to extract and identify the range of colours in natural dyes from dyed textiles, is not readily available.

- **Thin Layer Chromatography (TLC)** was used by many workers to identify various natural dyes in textiles. The dyes detected were based on insect and vegetable yellow, red and blue colour.
- **High Performance Liquid Chromatography (HPLC)**, which is a very useful tool to identify synthetic as well as natural dyes, was also used.



- **Infra-Red Spectral studies** were carried out to identify natural dyes viz., alizarin, indigo, dibromoindigo, saffron, weld, Persian berries and safflower yellow.
- While a non-destructive method was devised, for identifying faded dyes on fabrics through examination of their **Emission and Excitation Spectra**.
- **UV/Visible Spectroscopic studies** to identify a number of natural dyes were carried out by Schweppe, sobko and Chikh were carried out to identify madder, cochineal, indigo, etc., using different solvents for extraction.<sup>15</sup>

## **TECHNIQUES of IDENTIFICATION:-**

### **By Thin Layer Chromatography (TLC): -**

Thin layer chromatography is a microanalytical technique, which separates by adsorption, partition or ion exchange on a thin layer or film of adsorbent coated on a glass plate. This technique was introduced in 1938 by two Russian workers Ismailov and Shraiber, but only since 1958 has it rapidly gained popularity as a result of the work of Stahl, a German pharmacy lecturer, who developed, among other things, a spreading device to produce uniform layers of adsorbent on glass plates. He also standardized the preparation of suitable adsorbents to give consistent results. One of the typical terms used in TLC is

#### ➤ **Column Capacity Ratio (K'):**

K' value of solute is the usual method of indicating solute retention.

$$K' = (T_r - T_o) / T_o$$

Where,

$T_r$  = retention time of given peak

$T_o$  = retention time of unretained (solvent) peak

Use of K' values is preferred to simply quoting retention times, since latter can vary with flow rate variation from day to day, while K' remains constant.

K' values in HPLC are related to  $R_f$  values in TLC.

$$K' = (1 - R_f) / R_f$$

$$R_f = 1 / (1 + K')$$

$R_f$  = Ratio of distance traveled by solute to distance traveled by solvent front.  $K'$  value measures ratio of time spent by solute in stationary phase to time spent in mobile phase.

- The band of aqueous solution of the dye is applied on TLC plates by using TLC sampler. Development of the band is done in an ascending developing chamber with a mixture of different solvent in a required proportion as a solvent system. Plates dried using a drier and the bands are visualized in UV lamps and then scanned in a TLC scanner.

TLC studies are carried out on the extract of dyes using natural dye standards as reference. <sup>16</sup>

### **HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC): -**

It can separate a wide variety of compounds ranging in molecular weight from less than a hundred to upwards of few million. It is particularly applicable to the fractionation of high-molecular weight substances, thermally unstable compounds and products, which cannot be volatilized without decomposition.

**High performance liquid chromatography (HPLC)** is a powerful analytical tool and involves a hydrophobic, low polarity stationary phase that is chemically bonded to an inert solid such as silica. The process is conducted at a high velocity and pressure drop. The column is shorter and has a small diameter, but it is equivalent to possessing a large number of equilibrium stages. The separation is essentially an extraction operation and is useful for separating non-volatile components.

If two dyes have same  $R_f$  value, they are difficult to analyse by TLC. In contrast, dyes of different colours, which have the same retention time, may be quantitated with HPLC provided their spectra do not interfere. For example, many green dyes are mixtures of blue and yellow dyes and both dyes may be quantitated by HPLC even when they have the same retention time.

Example: - lycopene and  $\beta$ -carotene are determined on a Hewlett Packard 1050 HPLC, equipped with an autoinjector and UV-Visible Detector, Spherisorb column C18, 5 mm, 25 cm  $\times$  4.6 mm. a mixture of methanol, THF and water in a 67:27:6 ratio with a flow of 1ml/min (2  $\mu$ l injection volume) is used as the mobile phase; the detection is performed at 446 nm, corresponding to maximum of lycopene.

Lycopene and  $\beta$ -carotene were identified by comparing the retention time of the two pigments in the extraction mixture with those of their respective standard compounds. (Sigma products.)

Lycopene was also identified by comparing the UV-Visible,  $^1\text{H}$  NMR and Mass Spectra of the extracted substance with those of standard lycopene. The amount of total extractable pigment was determined after extraction with acetone: hexane for six hrs using a Soxhlet apparatus.<sup>12,17.</sup>

### **INFRA-RED SPECTROSCOPY: -**

IR spectroscopy is routinely used for identification of samples in the gas, liquid and solid states. Sample cells are made from materials, such as NaCl and KBr, that are transparent to IR Radiation. In comparison to other IR instruments, Fourier Transform Infra-Red Spectrometers provides for rapid data acquisition, in signal-to-noise ratio through signal averaging. In FTIR, the monochromator of IR instruments is replaced with a interferometer.

Example: -About 2mg of the purified dye sample is weighed in 298mg KBr pellets and the FTIR spectra are recorded between 4000 to 400  $\text{cm}^{-1}$  range on a Perkin Elmer system 2000FTIR.<sup>15,18,19.</sup>

### **UV-VISIBLE SPECTROSCOPY : -**

Absorption spectroscopy provides a useful tool for Qualitative Analysis, Identification of a pure compound by this method involves an empirical comparison of the details of the spectrum (maxima, minima and inflection points) of the unknown with those of pure compounds; a close match is considered to be good evidence of chemical identity, particularly if the spectrum of unknown contains a number of sharp and well-defined peaks. Dyes are extracted from the dyed textiles using DMF as solvent. The UV-Visible spectra of the extract are recorded with natural dye solution of DMF as reference standard. UV-Visible Spectrophotometer model: - Hitachi U-2000 and GBC UV/VIS 918.<sup>15.</sup>

### **DIFFERENTIAL SCANNING CALORIMETRIC STUDIES : -**

Differential Scanning calorimetric (DSC) studies are carried out to investigate the thermal behaviour of the natural dyes. Dye powder is directly used for DSC Studies. Identification of different natural dyes is carried out by comparing the details of endothermic and exothermic peaks from DSC Thermogram, and values of  $\Delta H$  for various natural dyes. DSC model: Mettler DSC 30. 15.

### **Analysis of Red-listed Chemicals: -**

The extracted dye is tested for the presence of Toxic Metals, Pesticides and Banned Aryl Amines by the methods prescribed for synthetic dyes.

**Heavy Metals:** - Wet Ashing of Extract and heavy metal content is determined by Atomic Absorption Spectroscopy (AAS).

**Pesticides:** - Extraction, clean up and Pesticides content is determined by Gas Chromatography/ Electron Capture Detector (GC/ECD), High Pressure Thin Layer Chromatography (HPTLC).

**Banned Aryl Amines:** -Reductive, cleavage, isolation and it is determined by Gas Chromatography/ Mass Spectroscopy (GC/MS.)

**Mordants:** -According to eco-standards, copper and chrome are red- listed and hence are not to be used. Problem posed by a mordant is that a substantial proportion of it is left unexhausted in the residual dyebath and may cause serious effluent problems. Aluminum and iron are relatively innocuous; they are abundantly available and produce excellent dyeings.<sup>20</sup>.

## **6. STANDARDIZATION of NATURAL DYES: -**

Dyes of natural origin are great for colour appreciation as any variation in the concentration of dye, mordant, types of water, soil and climate give variation in colours. In recent years, natural dyes are again getting importance due to harmful effects caused by synthetic dyes during their production and use.

### **Techniques of Standardization:-**

**Standardization of natural dye** should be done by conversion of natural dye extracts into a fine powdered, paste or solution form. Only water as such or at different pH and temperature would be

used for the colouring matter from the raw materials. Parameters such as pH, time and temperature of extraction should be worked out for optimization to achieve standardization.

Standardized natural dye should be pure and does not change appreciably due to atmospheric conditions. For the determination of standardized natural dyes accurately, it is necessary to compare it with some known standard of purity.

Standardization includes determination of extractive values using various solvent.

- Ash values: - Determination of ash value is useful for determining low-grade extracts.
- $R_f$  values: - Paper chromatography and Thin Layer Chromatography is widely used as a mean of assessing Quality and Purity.  $R_f$  values of natural dyes are determined under specific conditions and can be used for standardization by an aid to identification.
- Most of natural dyes are pH sensitive and they change colour with changing pH, use of buffered solution for extraction of the dye is first step towards standardization.
- Same natural colour extracts are not give the original colour of extract from the colour obtained on the fabric. Therefore, reproducibility of natural dye shade is very important in the process of standardization.
- Parameters such as pH, Temperature of extraction and conditions for getting the maximum colouring matters should be standardized.
- Considerable Standardization with respect to moisture, dye and ash content, particle size, the pH of dye storage stability should be done.

**Exact Measurement** is the key to Standardization.

**Standardization of Dye Extraction:** - The natural dye source (leaves, roots, woods etc) was collected, dried in shade and powdered sample is boiled in various solution medium (aqueous, alkaline, acid etc) in order to optimize the method of extraction. The optimizations are based on the optical density of the liquid, before and after dyeing using spectrophotometer and visual appearance.

**Optimization of Alkali / Acid concentration:** - Optimum alkali/acid concentration for 100 ml of water, for a pH to be standardized.

**Optimization of Extraction Time and Dyeing Time:** - Boiling for longer duration increases dye concentration. High dye yield is found after boiling for optimized time. High absorption is found for optimized dyeing time, above which dye depletion is observed.

**Optimization of concentration of Mordants:** - The concentration of mordants is optimized by considering the visual appearance and dye absorption.

**Optimization of Mordanting Methods:** - sometimes more than the mordant, the mordanting methods contributes towards the production of darker or lighter and faster shades.

**Project on Standardization of Eco-Friendly Natural Dyes: -**

**Alps Industries Limited.** was conceived and submitted a project for standardization of eco-friendly natural dyes to Technology Information, Forecasting and Assessment Council (TIFAC) a wing of Department of Science and Technology GOI (DST.) The total value of project was Rs. 4.25 Crores, out of which TIFAC was to give a lone of Rs. 2 Crores over a period of 3 years and the remaining contribution was to come from the Alps Industries Ltd. Project finally approved by the TIFAC under its Home Grown Technology scheme in July 1998. A highly competent team of Technologists from Academic Institutes and Industry are monitored the project.

A range of Do-It-Your-Self natural dye kits have been created by Alps Industries Ltd. Kit not only have the purified natural dye powder and required Auxiliaries and mordants but also have an instruction book with dyeing material and simple glasswares<sup>21, 22</sup>.

**Example: - Standardizing dyeing condition for African Marigold.**

**Method of Dye Extraction:** - known quantity of dye Marigold petals (African Marigold variety) were taken and soaked in cold water (MLR 1:40) overnight. The petals were boiled in the same water.

The Optimum **Time** for the extraction of dye was determined by boiling the dye material for 30, 45, 60 and 75 min.

The **pH** was recorded after dye extraction and then the pH was changed to acidic (pH 4) or alkaline (pH 10) by adding Acetic acid or Potassium hydroxide respectively, depending upon the medium which dyeing was to be carried out. Finally the dye extract solution was filtered and Optical Density was measured.

**Dye uptake:** - relation of wavelength ( $\lambda$ ). The dye extract was subjected to visual light of wavelength ( $\lambda$ ) using ELICO-SL 171 minispectrophotometer. The wavelength at which the optical density obtained was taken as the suitable wavelength, for the colour of the dye.



Percentage of dye absorption is calculated by

$$\% \text{ of Dye Absorption} = \frac{\text{Optical density before Dyeing} - \text{Optical Density after Dyeing}}{\text{Optical Density before Dyeing}} * 100.$$

**Dilution Factor:** -Dilution at which O.D. was around 0.3 was selected as a suitable dilution. Therefore, Dilution Factor for Marigold is 10.<sup>23</sup>.

Thus, the Standardization leads to perfection in natural dyed product with respect to overall Fastness Properties.

### **CONCLUSION: -**

- In recent years, an interest in natural dyes has been mainly manifested as Conservation and Restoration of old Textiles with replacement of synthetic dyes (which uses violent technology) by natural dyes for textiles, food, safety and so forth by using Mild Chemistry.
- The buyers from European Economic Community (EEC) Countries especially from Germany insist on a certificate for eco-friendliness of the commodities and therefore, Identification and Testing of natural dyes and natural dyed substrates as per Eco-standards as well as their authenticity has become imperative.
- The Research and Development work in Standardization of natural dyes are least or negligible. Very few serious attempts have been made to generate new information on the use of natural dyes. Most of the researches in this area are carried away by the empirical information reported in the literature that does not have any scientific reasoning or basis. Using raw materials for dyeing has many limitations. Besides its being of unknown composition, it generates considerable amount of biomass that is cumbersome to handle in

the dye-house. Non-availability of natural dyes, especially in the standardized form, which may be powder, paste or solution form.

- If there had been significant research on the use of natural dyes, it would be probable that they would be already being much more widely used than they currently are. As there is much catching up to do after 150 years of neglect, there is rapid scope for developments. This applies to the techniques of agricultural production and processing as well as to dyeing itself. A medium-to-long-term view of research and development is needed. It is unlikely that the total cost of R and D required to make natural dyes a profitable commercial reality need be very great when compared with many industrial products.
- Only small portion of a huge icebergs of standardization of natural dyes are only achieved. The contributions for standardization of natural dyes are made by some companies like Alps Industries Ltd. that uses Supercritical CO<sub>2</sub> plant for extraction of dyes for a step towards standardization. The standardized natural extracts are very much useful for Textiles, food, pharmaceuticals and cosmetics.
- It is a challenge for any enlightened dyer for working out suitable standardized applications of natural dyes on Textiles with eco-friendliness.

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***Dr. Ruma Chakrabarti is Associate Professor at Kumarguru College of Technology and A. Vignesh is Technical Compliance Manager in Hohenstein India Pvt. Ltd.***

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