Experimental Procedure

3.1 Material and Method

3.1.1 Raw material and Chemicals

All dye stuffs were easily available as many of them are waste products. The procurement of pomegranate rinds was done from juice shops, onion peels from the vegetable market and marigold flowers from local temples. Also the Dahlia flowers used in dye extraction were available in the campus (Department of chemical engineering, IT BHU).

All the solvents used (Ethanol, Methanol, Acetone and Hexane) were of reagent grade and supplied by Merck. Sodium carbonate, Hydrochloric acid, sodium hydroxide, alum and tin chloride were supplied by SD Fine. Labolene were used as non-ionic soap for washing of yarns and purchased from local suppliers.

Enzymes Cellulase and Pectinase were purchased from Varuna Bio cell, Industrial area, Ramnagar, Varanasi.

Bleached cotton and wool yarns were procured from one of the suppliers to the carpet industry of Bhadohi and Varanasi.

3.1.2 Instruments

The basic infrastructure required for this work was provided by research lab in Department of chemical engineering IT BHU. Tray drier, ball mill, weighing balance, ultrasonic bath, heating mental, reflux condenser, orbital shaker, Rotavapour, vacuum filtration unit and spry drier machines were used during this work and all were available in laboratory. Some analytical instruments viz UV-Visible spectrophotometer, HPLC, SEM, FTIR were also used. UV-Visible
spectrophotometer and HPLC were available in laboratory, whereas SEM and FTIR facilities were provided by other departments.

3.1.2.1 Tray Drier

The term tray drier is normally applied to the system with some form of air heater and a fan to pass air over the material being dried. The driers are made of trays held in a cabinet which is connected to a source of air heated by gas, diesel or bio-mass. The air temperature is usually controlled by a thermostat which is normally set between 50 and 70 °C. The air enters the bottom of the chamber below the tray and rises through to tray of material being dried, and exits from an opening in the top of the chamber. ([en.wikipedia.org/wiki/Tray_drier](en.wikipedia.org/wiki/Tray_drier))

For the storage of dye stuffs before the extraction process the plant materials were dried in tray drier at 50°C for 24 hours, in order to remove moisture from them.

![Tray Drier](image)

**Fig. 3.1: Tray Drier**

3.1.2.2 Ultrasonic Bath

Sonication is the act of applying sound energy to agitate particles in a sample for various purposes. In the laboratory it was usually applied using an ultrasonic bath.

Ultrasonic waves travel through material media leading to successive expansion and compression cycles. Expansion cycles produce negative pressure in a
liquid which causes formation of cavities in liquid phase. In spite of occurrence of bubbles nucleation during the expansion cycles of ultrasonic waves, in these cycles the cavitation nucleus growth, since molecules of vapour and gases migrate to cavities. On the other hand, for compression wave cycles, a fraction of gases and vapour molecules can be expelled from the bubble diameter allows a critical value when it collapses leading to a local temperature and pressure higher than 5,000 K and 1,000 atm, respectively. (en.wikipedia.org/wiki/Sonication)

The phenomenon associated to the nucleation, growth, and collapse of bubbles submitted to ultrasonic waves is named as cavitation process. During the cavitation process, volatile molecules inside the bubbles are preferentially mineralized by thermal mechanism while hydrophilic molecules are decomposed by free radicals in the cavitation bubbles neighbourhoods. A particular situation occurs in a medium containing suspended particles. In these cases, asymmetric collapse originate high speed micro jet towards the solid erosions and cleavages of solid material lead to an increase of solid surface area, thus improving the extraction of materials from solid samples irradiated with ultrasonic waves.

In this work ultrasonic bath is used in UAE process. Extractions were performed in an Ultrasonic bath with frequency 27-30 MHz and were working at 160 V.

Fig. 3.2 : Ultrasonic Bath
3.1.2.3 Rotary–evaporator (Rotavapour)

A rotary evaporator is a device used for the efficient and gentle removal of solvents from samples by evaporation under reduced pressure. Rotavapour is used for the concentration of extracts obtained after the vacuum filtration and for the recovery of solvent.

The main components of a rotary evaporator are:

1. A motor unit that rotates the evaporation flask or vial containing the sample.
2. A vapour duct that is the axis for sample rotation, and is a vacuum-tight conduit for the vapour, being drawn off of the sample.
3. A vacuum system, to substantially reduce the pressure within the evaporator system.
4. A heated fluid bath (generally water) to heat the sample.
5. A condenser with a coil passing coolant (Water).
6. A condensate-collecting flask at the bottom of the condenser to catch the distilling solvent after it recondenses.
7. A mechanical or motorized mechanism to quickly lift the evaporation flask from the heating bath.  

(\textit{en.wikipedia.org/wiki/Rotary-evaporator})

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{rotavapour}
\caption{Rotary–evaporator (Rotavapour)}
\end{figure}
3.1.2.4 Spray Drier

Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas. This is the preferred method of drying of many thermally-sensitive materials. Spray dryer takes a liquid stream and separates the solute or suspension as a solid and the solvent into a vapour. The solid is usually collected in a drum or cyclone. The liquid input stream is sprayed through a nozzle into a hot vapour stream and vaporised. Solids form as moisture quickly leaves the droplets. (en.wikipedia.org/wiki/Spray_drier)

![Fig. 3.4 : Spray Drier](image)

3.1.3 Analytical Instruments

3.1.3.1 UV-Visible Spectrophotometer

The ultraviolet-visible (UV-VIS) adsorption spectra of extracted dyes were recorded, on Systronics, PC Based Double Beam Spectrophotometer 2202. UV Visible spectroscopy measures the response of a sample to ultraviolet and visible range of electromagnetic radiations. Organic compounds, especially those with high degree of conjugation also absorb light in the ultraviolet or visible region of the electromagnetic spectrum. In this region of electromagnetic spectrum, molecules undergo electromagnetic transition. Molecules containing \( \pi \) -electrons or non -
bonding electrons can absorb the energy in form of ultraviolet or visible light to excite these electrons to higher anti-bonding orbitals. The more easily excited the electron the longer the wavelength of light it can absorb. The wavelength of absorption peaks can be correlated with the types of bond in a given molecule and are valuable in determining the functional groups within the molecule.

The spectrum alone is not however, a specific test for a given sample. The nature of the solvent, pH of the solution, temperature, high electrolyte concentration, and the presence of interfering substance can influence the absorption spectrum

![Fig. 3.5: UV-Visible Spectrophotometer](image)

**Beer-Lambert Law**

The method is used in a quantitative way to determine the concentrations of an absorbing species in solution using Beer- Lambert law.

\[
A = \log_{10} \left( \frac{I_0}{I} \right) = \varepsilon cl
\]

Where A is the measured absorbance, I₀ is the intensity of the incident light at given wavelength, I is the transmitted intensity, l is the path length through the sample and c is a constant called as molar extinction coefficient or molar absorptivity.
3.1.3.2 High-Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a technique in analytical chemistry used to separate components in a mixture, to identify each component and to quantify each component. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent. The active component of the column, the sorbent is typically a granular material made of solid particles 2-50 micrometres in size. The components of the sample mixture are separated from each other due to their different degree of interaction with sorbent particles. The pressurized liquid is typically a mixture of solvent (Water, acetonitrile and/or methanol) and is referred as mobile phase.

The schematic of a HPLC instrument typically includes a sampler, pumps and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pump delivers the desired flow and composition of the mobile phase through column. A detector generates a signal proportional to the amount of sample component emerging from the column. A digital microprocessor and user software control the HPLC instrument and provide data analysis. (en.wikipedia.org/wiki/High_performance-liquid-chromatography)

![Figure: 3.6 : HPLC](image-url)
3.1.3.3 Scanning Electron Microscopy (SEM)

Scanning electron microscope is a type of electron microscope that produces images of a sample by scanning it with focused beam of electrons. The electron interact with atoms in the sample, producing various signal that can be detected and that contain information about the sample’s surface and composition. SEM can achieve resolution better than 1nm.

3.1.3.4 Fourier Transform Infrared Spectroscopy (FTIR)

IR spectroscopy is one of the most common spectroscopic techniques, used to determine the structure of organic compounds. The main objective of this analysis is to identify the different functional groups present in an organic molecule. Each functional group absorbs characteristic frequency of IR radiation.

Experiments

All the dye stuffs viz pomegranate rind, marigold flowers, red onion rind and dahlia flowers were collected; petals of the flower were removed from calyx and were washed thoroughly under the tap water and then dried in air. After washing all plant materials were dried in tray-drier. Dried pomegranate rinds and red onion peels were then de-sized in ball mill. Dried marigold and dahlia flower’s petals were used for extraction as intact. After pre-treatments various experiments were performed for the extraction of dyes.

3.2 Extraction Processes

Four different extraction processes were followed in this work. Throughout all the extraction processes, M: L ratio 1:10 was used for pomegranate dye extraction, 1:15 for red onion dye and 1:20 for marigold and dahlia dye extraction.
3.2.1 Solvent Extraction (Conventional Extraction Process)

In solvent extraction process, the dried dye stuffs were mixed with solvent in a 500 ml reflux apparatus. Required pH was maintained and extraction process was then carried out at different temperatures from 30°C-80°C (Here temperature 30°C referred to room temperature and for extraction at 40°C experiments were performed in an orbital shaker maintained at required temperature) for 15-90 minutes. After filtration, concentration and drying of extracts, yields were compared for optimal temperature and time for solvent extraction.

3.2.2 Ultrasound Assisted Extraction

Standard extraction protocol was used using a 500 ml beaker containing dried dye stuffs and solvent. The beaker was covered using aluminium foil to prevent solvent evaporation, after maintaining the pH the beaker was immersed into the ultrasonic bath with frequency 27-30 MHz working at 160 V under a controlled water level at about 2-3 cm from the bottom of the bath. Extractions were performed for different extraction time and at different temperatures (which were maintained by using hot water and ice cubes). Yields were compared to get the optimal extraction time and temperature for the extraction under UAE.

3.2.3 Enzyme Assisted Extraction

In EAE the dried plan material were sprayed with selected enzymes viz; Cellulose, Lignase, Xylanase and Pectinase (depending on the composition of dye stuffs). The enzyme treated material was washed with little amount of distilled water and then this material was transferred in 500 ml round flask and appropriate amount of solvent was poured into it. After maintaining the required pH level, the contents of the conical flask were subjected to solvent extraction at various temperatures. Extraction was also optimized to get the optimal concentration of enzymes and optimum incubation time.
3.2.4 Enzyme Mediated Ultrasound Assisted Extraction

EUAE is the process where the dye stuffs were treated with enzymes, and then after washing with little amount of distilled water the material was transferred in a 500 ml beaker and the solvent was poured into it. After maintaining the optimum pH, UAE was done at various temperatures to see the effect of temperature on EUAE.

3.3 Concentration and Drying of Extracted Dye

After extraction, the extracts were cooled to room temperature and filtered through Whatman No.1 paper under vacuum. The filtered liquid was vacuum evaporated in a rotary vacuum evaporator to about half of the original volume. Before the drying of extracted dye via spray drier we need to concentrate the extract to recover the solvent and to get the optimum concentration of extracts for spray drying. (If we used a dilute solution for spray drying it could result in a sticky mass instead of free flowing powder form of dyes). Concentrations of extracts were done in a rotavapour at 70°C temperature for pomegranate dye, where water is the solvent and at 50°C for other dyes containing organic solvents with low boiling temperatures, and at rotation speed of 140 rpm.

This concentrated dye extract was spray dried in Spraymate (Jay Instruments and Systems Pvt. Ltd. TTC Industrial Area, Turbhe, Navi Mumbai). The inlet and outlet temperatures of spray drier were maintained at 140°C and 80°C, respectively and aspiration speed was 1200, while flow rate was 35 rpm. The dried powder was collected, stored in a desiccator and used as dye without further purification. All experiments were carried out in triplicate and the percent gravimetric yields (%w/w) have been reported as an average of three values obtained. The spectrophotometric yields reported above were found to be higher (2-4%) than that of gravimetric yields.
owing to the loss of dye in the chamber, cyclone and vacuum chamber cloth of the Spray Drier.

3.4 Dyeing with Extracted Dyes

The wool yarns, which are generally used in textiles and carpet industry, were selected for dyeing. The pre-mordanting method was used in the present investigation.

3.4.1 Scouring of Cotton and wool Yarns

Wool and cotton yarns were washed with a solution containing 2.0g/L of non-ionic detergent (labolene) at 40-45°C for 30 minutes, keeping the material to liquor ratio at 1:50. The scoured material was thoroughly washed with tap water and dried at room temperature. The scoured material was soaked in clean water for 30 minutes prior for dyeing or mordanting.

3.4.2 Mordanting

Pre-mordanting with metals salts were carried out before dyeing. The mordants were dissolved in water to make 2% solution of mordant with material to liquor ratio 1:50, and then it was brought to heating. Temperature of dye bath was raised to 60°C and over half an hour and left at that temperature for another 30 minutes. Mordanted material was then rinsed with water thoroughly, squeezed and dried.

3.4.3 Dyeing

Dye bath was prepared by required amount of dye solution with M: L ratio 1:15 then 5.0 g of mordanted wool yarns were put into dye solution at room temperature (25°C). The temperature was then raised to 90 °C and dyeing continued for another 45 min. After dyeing, cold wash was given with water followed by washing with soap solution. Finally, dyed yarns were washed several times with tap water and dried in air.
3.5 Fastness studies

Fastness properties of the dyed yarns were evaluated at Indian Institute of Carpet Technology, Sant Ravidas Nagar, Bhadohi, UP. The details of the values assigned for these properties are:

5 = Negligible (Excellent)
4 = Slightly changed (Good)
3 = Noticeable changed (Fairly good)
2 = Considerably changed (Fair)
1 = Much changed (Poor)

3.6 Evaluation of L, a*, b* and K/S

After dyeing colour strength were carried out to have an idea about the dye uptake. L, a and b form the three perpendicular axes of the colour space. In this work L, a, b and K/S values have been measured by Gretag Macbeth, colour –eye 7000A machine, at Textile department in delhi IIT.

3.7 UV-Visible spectra of Dyes

The ultraviolet-visible (UV-VIS) adsorption spectrum was recorded, on Systronics PC Based Double Beam Spectrophotometer 2202, over the range of 200-800 nm, peaks were obtained at:

- 424 nm and 460 nm for pomegranate dye,(Figure 6.1.)
- 474 nm for marigold dye, (Figure 6.2.)
- 368 nm and 526 nm for red onion dye,(Figure 6.3.)
- 281nm and 502 for dahlia dye.(Figure 6.4.)

To estimate the yield at different time intervals of 10 minutes, during the course of extraction 1.0 mL liquid was pipetted out and was diluted to make 10.0 mL
volume. This solution was centrifuged at 2000 rpm to remove suspension. Intensity of extracted colour was measured by spectrophotometer at particular wavelength and then the extent of extraction was monitored by using a calibration curve drawn by dissolving the dye powder in water.

3.8 HPLC of Dyes

HPLC analysis was carried out to determine the dye uptake by yarn. The chromatograph was equipped with UV Visible detector and C18 column manufactured by Cecil Instrument.

The crude extracted liquid was filtered through 0.22 µm pore size injection filter. A 20 µL-filtered sample was injected into the column for HPLC analysis. The mobile phase consisted of 70% Acetonitrile and 30% Ethanol. The flow rate was 1 mL per minute, and the spectral measurement was made at the wavelength range 370 nm for red onion dye and 474 for marigold dye. The HPLC of the dye bath was taken in order to find out the component responsible for the dyeing the fabric, before and after the dyeing. For this purpose, 10 mL of the colorant solution from the dye bath, before and after dyeing, was taken and its volume was made to 100 ml. This solution was used for HPLC analysis after filtration through 0.22 µm pore size injection filter, in order to have an idea about the amount of dye attached to the fibre.

3.9 FTIR of Dyes

The FTIR of the dye was recorded on Varian 3100 in KBr with Thermo Nicolet 5700 Spectrometer.

3.10 SEM

The Scanning Electron Micrograph (SEM) of red onion peels and marigold petals, before and after extraction was taken on FEI-QUANTA 200F.