Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather

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1. Introduction

There is a growing demand for eco-friendly/non-toxic colorants, specifically for health sensitive applications such as coloration of food and dyeing of child textile/leather garments. Recently, dyes derived from natural sources for these applications have emerged as an important alternative to potentially harmful synthetic dyes and pose need for suitable effective extraction methodologies. The present paper focus on the influence of process parameters for ultrasound assisted leaching of coloring matter from plant materials. In the present work, extraction of natural dye from beetroot using ultrasound has been studied and compared with static/magnetic stirring as a control process at 45 °C. The influence of process parameters on the extraction efficiency such as ultrasonic output power, time, pulse mode, effect of solvent system and amount of beetroot has been studied. The use of ultrasound is found to have significant improvement in the extraction efficiency of colorant obtained from beetroot. Based on the experiments it has been found that a mixture of 1:1 ethanol–water with 80 W ultrasonic power for 3 h contact time provided better yield and extraction efficiency. Pulse mode operation may be useful in reducing electrical energy consumption in the extraction process. The effect of the amount of beetroot used in relation to extraction efficiency has also been studied. Two-stage extraction has been studied and found to be beneficial for improving the yield for higher amounts of beetroot. Significant 8% enhancement in % yield of colorant has been achieved with ultrasound, 80 W as compared to MS process both using 1:1 ethanol–water. The coloring ability of extracted beet dye has been tested on substrates such as leather and paper and found to be suitable for dyeing. Ultrasound is also found to be beneficial in natural dyeing of leather with improved rate of exhaustion. Both the dyed substrates have better color values for ultrasonic beet extract as inferred from reflectance measurement. Therefore, the present study clearly offers efficient extraction methodology from natural dye resources such as beetroot with ultrasound even dispensing with external heating. Thereby, also making eco-friendly non-toxic dyeing of fibrous substances a potential viable option.

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discharges, which may pose health hazards, viz., carcinogens. Our earlier work focused towards, improving dye uptake by substrates leading to near zero discharge levels with ultrasound in leather dyeing process in detail [4–9] as in-plant control measure rather than end of pipe treatment. Influence of ultrasound in leather processing has been analysed and reviewed in detail as eco-friendly methodology [4]. Our recent paper analyses sono-technology as a green method of activation in leather processing [10]. However, growing environmental concern with regard to synthetic dyes, natural dyes offer scope for eco-friendly way of dyeing of fibrous materials such as textiles or leather and for food coloration.

Natural dyes are non toxic and non allergic which are very important for some sensitive applications. The process of release of potential toxic chemicals from substrates during usage attracts worldwide attention such as EU and REACH. There is a growing need for the non-toxic method of coloration for health sensitive applications such as children textile/leather garments as well as toys and for food coloration. Coloration of child garment/toys require a special attention due to the reason that kids have the habit of biting these materials which cause release of the colorant and intake leading to possible health risks. Therefore, there is a need for suitable coloring material from natural vegetation sources and efficient extraction methodology for the same.

1.1. Beetroot as a natural dye source

There is a pressing need for dyeing of fibrous substances such as leather, and textiles for some health sensitive applications. Betalain colorants extracted from red Beetroot (Beta vulgaris) provide a natural alternative to synthetic dyes [11]. Beetroot was selected as the topic of the present study because of the good colorant yield and the prominent peak it gave in the visible region of the spectrum for better quantitative analysis. Beetroot colorants are commercialized as juice concentrate and FDA classifies them as vegetable juices, which are commonly spray dried with maltodextrin to obtain beetroot powder [12]. Most varieties of Beetroot contain betalains-red colored cyanins and betanins and yellow colored xanthins. Cyanin represents 75–90% of total color present in beetroot. Betanin is the most prevalent betalin in red beets, which typically contain large quantities of it (e.g. 300–600 mg/kg). According to studies conducted it has been found that 100 g of raw beetroot contain 87.1 g of water, 7.6 g of carbohydrate, 1.7 g of protein and 0.1 g of fat [13]. Betalain, natural food colorant is associated with the antioxidant, antiviral and antimicrobial activities [12]. Therefore, beetroot dye is associated with nutrient value along with non-toxic nature suitable for dyeing application where health aspect is a prime criterion. Beetroot (B. vulgaris) is one of the richest sources of betanin and used for imparting a desirable red color. Unlike the synthetic dyes, these beetroot based natural dyes are eco-friendly and pose no environmental problems.

Extraction of coloring matter from beetroot is a solid–liquid leaching process involving mass transfer problem. Since the coloring matter is strongly bound with plant cell membranes, extraction could be better by way of some improved methods such as ultrasound. The gamma ray irradiation technique studied to improve the extraction efficiency led to possible degradation and instability of coloring matter [12]. The use of electric pulse studied for the same [14] may involve operational difficulties. Therefore, the use of power ultrasound to improve the extraction of beetroot dye and application to the substrates such as leather has been studied for the first time and reported in this paper.

1.2. Power ultrasound – sonochemistry

Ultrasound may be broadly classified according to frequency range as power ultrasound (20–100 kHz) and diagnostic ultrasound (1–10 MHz). When a liquid is irradiated by ultrasound, micro bubbles appear, grow and oscillate extremely fast and even collapse violently if the acoustic pressure is high enough. The occurrence of these collapses near a solid surface will generate micro jets and shock waves. Moreover, in the liquid phase surrounding the particles, high micro mixing will increase the heat and mass transfer and even the diffusion of species inside the pores of the solid [10,15]. Our recent studies show significant improvements in solid–liquid myrobalan tannin extraction process due to the use of ultrasound [16].

1.3. Objectives of the present study

The specific objectives of the present study include,

(i) To study the influence of ultrasound on the extraction of natural dye from beetroot compared to magnetic stirring as control process.
(ii) Study the influence of process parameters for extraction such as ultrasonic output power, time, pulse mode, and effect of the solvent system.
(iii) Scale-up possibility study with amount of beetroot as well as two-stage extraction.
(iv) Application beetroot dye extracted with ultrasound for coloration of leather as well as sheet of paper for health sensitive applications.

2. Experimental section

2.1. Experimental setup

Ultrasound extraction experiments (US) were performed using ultrasonic probe (VCX 400, Sonics and Materials, USA, 20 kHz and 0–400 W) in a glass vessel with provisions to set required output power time and temperature [16]. Control experiments were performed with a magnetic stirrer (MS), which had provision to control the temperature. Beetroot (B. vulgaris) grown in agriculture land in Southern part of India has been procured from Chennai based vegetable market and used for the experiments. Distilled water and Ethanol (AR reagent, SD fine chem. Ltd., India) have been used for the experiments.

2.2. Extraction of beet-dye with ultrasound

Freshly cut cubical beetroot pieces of 5 mm dimension were used for the experiments. Typically, 1 g of sample with 50 ml distilled water was taken in the extraction beaker. The beaker was covered using aluminum foil to prevent loss of solvent by evaporation. The ultrasonic probe was placed in the beaker with pre-selected values of time and power output. The US bath temperature was maintained at around 45 °C in order to prevent potential heat damage to the plant material. Extraction with MS was carried out at 45 °C using thermostat in the stirrer as control experiment for comparison. Hence from here onwards in this paper the term ‘US’ refers to ultrasonic extraction without any external heating and ‘MS’ refers to magnetic stirring extraction with external heating both maintained at 45 °C. Samples were taken at every 30 min and the optical density (OD) values were recorded with the help of UV–VIS spectrophotometer. After the extraction time of 3 h, yield and extraction efficiency have been calculated by gravimetric method. The extract was stored at low temperature for future reference.

All the experiments were repeated for duplicate and the average of extraction data was used in plotting the graphs. Standard error has been calculated for the data.
2.3. Dyeing of leather

In order to find out the applicability of natural dye from beet-root by US method, leather dyeing experiments were carried out. Beetroot natural dye was extracted typically from 10 g of freshly cut pieces in presence of ultrasound, 80 W with 100 ml of water as solvent. Similar extraction was also carried by MS method for comparison. Volume of dye solution for leather dyeing experiment has been calculated so as to give 8% dye (based on the leather weight). Chrome tanned wet-blue goat leather was cut into 9-cm diameter circular pieces as per SLTC official method[17] of sampling and neutralized to pH 6.0–6.5 using 1% sodium formate and 1% sodium bicarbonate solution. Dye solution along with neutralized leather piece was taken in a 1000 ml beaker. Leather dyeing was carried out separately in two beakers with magnetic stirring for 7.5 h. The dye sample was taken from the dye bath for every 30 min to calculate the uptake of dye in leather. Fastness properties of the dyed leathers were also studied as per SLTC official method. Dye ability of ultrasonic and magnetic stirring extracts was also compared in the dyeing of paper. Influence of ultrasound, 80 W in natural dyeing experiments in leather was also carried and compared with magnetic stirring.

2.4. Analytical methods

2.4.1. UV–visible spectrometric analysis

In order to perform the quantitative analysis of the beet dye colorant, wavelength of maximum absorbance ($\lambda_{\text{max}}$) for the beetroot extract was analysed using Shimadzu UV–visible spectrophotometer UV-2101 PC. The UV–visible spectrum of Beetroot dye extract is shown in Fig. 1. Two peaks corresponding to (i) Betaxanthin – 480 nm and (ii) Betanin – 530 nm have been obtained. The schematic representation of these compounds is given as Schemes 1 and 2 respectively. Absorbance values at the wavelength $\lambda_{\text{max}}$ – 480 nm for the samples collected from the experiments have been recorded. Then the amount of dye present in the extract was calculated from the calibration graph drawn for the beetroot dye as shown in Fig. 3. From the calibration graph, a linear fit with equation $Y = 0.501 \times X$ was obtained (Fig. 1). Where, $Y$ – absorbance value at 480 nm and $X$ – concentration of colorant in mg/L. The colorant concentration in the extract (in mg/L) was calculated using the formula $Y/0.501$.

2.4.2. Gravimetric analysis

In order to find out the total extract from beetroot, samples were taken after the extraction from both ultrasound and control extracts were filtered and taken in a clean, dried and weighed glass dishes. The extracts were dried in a hot-air oven until all the water evaporated. The dishes were then cooled in a desiccator and weighed. The drying, cooling and weighing procedure was repeated to get the constant weight and the weight of the extract was determined. The weight of the extract obtained per gram of the beetroot used was calculated. The yield for total extract was calculated using the formula as follows:

$$\% \text{ Yield of total extract} = \frac{\text{Total Extract obtained (g) \times 100}}{\text{Amount of beetroot used (g)}}.$$ 

The yield for colorant obtained was calculated using the formula as follows:

$$\% \text{ Colorant yield} = \frac{\text{Colourant obtained (g) \times 100}}{\text{Amount of beetroot used (g)}}.$$ 

2.4.3. Maximum extractable material (MEM)

Experiments were performed to find out the MEM from the beetroot in conventional method. One gram of beetroot was soaked in 50 ml of 1:1 water–ethanol mixture in a clean glass beaker and maintained at 50 °C with MS. The beaker was tightly covered with aluminium foil to prevent the evaporation of water from the beaker. The OD values were monitored till there was no appreciable change, i.e. 15 h. The colorant yield after 15 h extraction was

![Scheme 1. Betaxanthin.](image1)

![Scheme 2. Betanin.](image2)
calculated and taken as MEM. The extraction efficiency of a process can be calculated using the formula as follows:

\[
\text{Extraction efficiency (\%)} = \left( \frac{\text{Colorant yield}}{\text{MEM Colorant yield}} \right) \times 100
\]

The enhancement due to ultrasound in \% colorant yield as compared to MS process has been calculated using the formula as follows:

\[
\text{Enhancement in \% yield of colorant} = \left( \frac{\% \text{ Colorant yield for (US – MS)}}{\% \text{ MEM Colorant yield}} \right)
\]

2.4.4. Color measurement analysis

Quantification of color of the beet color dyed leather as well as paper was made according to the Commission Internationale de l’Eclairage (CIE) system of color measurement with 10° standard observer data [1]. \(L^*, a^*, b^*\) values for both the grain side of the dyed leathers were obtained using Milton Roy color mate HDS spectrophotometer as described earlier [5]. The variables \(L^*, a^*, b^*\) are the variables in the CIELAB color space and explained as follows.

More negative value of \(L^*\) denotes more darker shade and more positive value of \(L^*\) for more light shade of the color. More negative value of \(a^*\) implies more green color and more positive value of \(a^*\) for more red color. More negative value of \(b^*\) means more blue color and more positive value of \(b^*\) for more yellow color.

2.4.5. Analysis of spent dye liquor in leather dyeing process

The spent dye liquor samples were collected at regular intervals of time from the dye bath and analysed for the dye content. The \% dye exhaustion was calculated using the formula as follows:

\[
\text{% Beetdye exhaustion} = \frac{\text{Beetdye offered, g} – \text{Beetdye in spentliquor, g}}{\text{Beetdye offered, g}} \times 100
\]

3. Results and discussions

3.1. Maximum extractable material

The colorant yield in the extract was calculated from the MEM experiments. There was no appreciable change in the colorant yield value after 15 h. The results indicate that 0.19 g of extract can be obtained from 1 g of beetroot when ethanol water mixture is used as solvent as shown in Fig. 6. Whereas, 0.17 g was the MEM when water was used as the solvent for extraction as shown in Fig. 2.

The standard error in the beetroot colorant extraction data has been found to be maximum of 3 mg/g of beetroot used and 6 mg/g for the total extracts obtained in various experiments.

3.2. Effect of ultrasound in dye extraction

The results indicate that there is a significant improvement of 7 and 1.4 fold in beet dye extraction using ultrasound, 80 W as compared to static and magnetic stirring respectively as shown in Fig. 2 with water as solvent. However, there is a gap in the ultrasonic 80 W processes as compared to MEM with water.

3.3. Effect of solvent and its ratio on extraction

Experiments were conducted using ethanol–water mixture as solvent for beetroot dye extraction. Ethanol was selected as solvent because of its polarity and less hazardous in nature. Different solvent ratios were used for the extraction purposes. Ultrasound, 80 W was also used to the extraction and the results were compared. It was seen that ethanol–water mixtures gave better results than use of water alone as shown in Fig. 3. The extraction in the presence of ultrasound for ethanol water ratio of 1:1 gives the maximum colorant yield.

3.4. Effect of extraction time

The kinetics of extraction with ultrasound as compared to control processes is shown in Fig. 4. The order of colorant yield for various processes indicates, Ultrasound, 80 W (1:1 water–ethanol) > Magnetic stirring (1:1 water–ethanol) > Static control.
The results indicate that rate of extraction is faster during initial 1 h period and subsequently remain with marginal increase for 3 h process time, for all these processes.

3.5. Effect of ultrasonic output power

The influence of various ultrasonic power 40–120 W on extraction has been studied. The total extract yield as well as colorant yields for the different power outputs are shown in Fig. 5. The kinetics of extraction for different ultrasonic power is shown in Fig. 6. According to the results, the total yield of extract is maximum at 100 W power output, whereas colorant yield at 80 W is similar to 100 W. Moreover, there is excessive energy dissipation in the form of heat when 100 W is applied, which may lead to degradation of substance and hence requires cooling. Considering these, 80 W of power output from the sonicator is better suited for the extraction of the beetroot dye under the given process conditions. Therefore, the extraction is found to be effective at 80 W ultrasonic power and matches with the MEM with 1:1 ethanol–water solvent mixture as shown in Fig. 6. The stability of the beetroot colorant was found to be good and there is no adverse damage due to the ultrasonic intensity employed up to 100 W under the given process conditions.

3.6. Effect of ultrasonic pulse mode

In order to reduce the electrical energy consumption, experiments were conducted with ultrasonic pulse mode operation of 1 s ON 1 s OFF with 1:1 ethanol–water. Interestingly, ultrasonic pulse mode is found to be better in terms of colorant yield as compared to continuous mode as shown in Fig. 7. Hence pulse mode operation of ultrasound is found to increase the extraction efficiency as well as the energy efficiency of the process. The reason for the improvement in extraction for ultrasonic pulse mode may be due to non steady state mass transfer operative as compared to continuous mode.

3.7. Effect of amount of beetroot – multistage extraction: Ultrasound influence

Experiments were carried out to find the effect of the amount of beetroot used in relation to extent of extraction efficiency. The amount of beetroot varied from 1 to 8 g with 50 ml of 1:1 ethanol–water solvent system using ultrasound, 80 W. The total colorant in the extract obtained from beetroot increase with amount of material used as shown in Fig. 8 for two-stage extraction process. The extraction efficiency and overall colorant yield for the two stages for both MS and US processes are given in Table 1. The trend indicates extraction capability for 80 W ultrasound power even with higher amount of beetroot. Second stage extraction becomes relevant and important for getting total better yields when higher amount of beetroot is used.
amounts of beetroot were used for the given ultrasonic power under the given process conditions. Therefore, ultrasonic power has to be selected according to the amount of materials used in order to get better yields. The results also indicate that there is a sort of linear relation \( Y = mX + b \) for total extract obtained with amount beetroot used from 1 to 8 g as shown in Fig. 8. The linear regression equation for ultrasound extraction was found to be \( Y = 0.1001 \times X + 0.03 \) as compared to \( Y = 0.0895 \times X + 0.01 \) for the magnetic stirring control. MEM results for colorant from beetroot using 1:1 ethanol–water show, 19% yield. Based on this maximum yield and experimental data from Table 1, significant 8% enhancement in % colorant yield could be achieved with US for 8 g beetroot as compared to MS process. This enhancement in extraction with ultrasound could be attributed to the ultrasonic effects such as micro jet formation and acoustic streaming other than bulk raise in temperature as both the processes were carried out at same temperature of 45 °C Table 2.

3.8. Leather dyeing: Applicability of beetroot extract

Leather dyeing experiments were carried out for 7.5 h using beetroot natural dye extract obtained with ultrasound, 80 W and compared with that of magnetic stirring method. The photograph of the beetroot extracts obtained by the two methods is shown in Fig. 9, which shows better color intensity for US extract. The photograph of the dyed leathers with beet dye extract is shown in Fig. 10.

3.9. Ultrasound assisted natural dyeing of leather

In order to improve the uptake of beetroot dye in leather, use of ultrasound, 80 W in natural dyeing process has been studied with US beet dye extract and compared in magnetic stirring dyeing. The dye concentration in the dye bath was monitored and related to dye intake by the leather sample. It was found that beet dye intake in leather was more in the case of ultrasonic dyeing as compared to magnetic stirring during the course of the dyeing process as shown in Fig. 11. Therefore, ultrasound is found to be beneficial in natural dyeing of leather with improved rate of exhaustion. Spent dye liquor from the dye bath for ultrasound aided process is less colored as compared to magnetic stirring process after 7.5 h dyeing. The photograph of the spent dye liquor is shown in Fig. 12. The dye exhaustion was found to be 62% on ultrasonic assisted dyeing and 49% on magnetic stirred dyeing.

3.10. Color analysis by reflectance measurement

Quantification of color value of the leathers dyed (by stirring method) with natural beet dye obtained from both US and MS methods was analysed by reflectance measurement. The color

Table 1

<table>
<thead>
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<th>S.No</th>
<th>Amount of beetroot used (g)</th>
<th>Ultrasound, 80 W Extraction efficiency (%)</th>
<th>% Colorant yield stage (I + II)</th>
<th>Magnetic stirring Extraction efficiency (%)</th>
<th>% Yield stage (I + II)</th>
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<td>II Stage</td>
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<tr>
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<td>6</td>
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<td>10.5</td>
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</tr>
<tr>
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<td>7</td>
<td>42.1</td>
<td>12.8</td>
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<td>10</td>
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<td>16.5</td>
<td>58.6</td>
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</table>

%Yield out of 19% as maximum yield from MEM.
values of the samples $L^*$, $a^*$, $b^*$, $c$, $h$ with difference $\Delta L$ and $\Delta a$ are shown in [2]. There is a significant improvement in the color intensity and red color (color corresponding to beet dye) for leather as well as paper dyed with US beet extract as compared to MS method as inferred from $\Delta L$ and $\Delta a$ values. The photographs of the dyed sheets of paper for the two experiments are shown in Fig. 13.

### 3.11. Fastness properties of dyed leather

Fastness properties of the leathers dyed (by stirring method) with natural beet dye obtained from both US and MS methods were analysed by adopting SLTC official test method [17]. The results indicate that dry fastness value for both US and MS beet extract is excellent and found to be comparable. However, the value for wet rub and perspiration resistance is poor, which is not unexpected. The reason is due to the fact that mordant was not employed during the natural leather dyeing process. It is a

<table>
<thead>
<tr>
<th>S. No</th>
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**Fig. 9.** Extracted beet dye samples. Left: with ultrasonic extract; Right: with magnetic stirring extract.

**Fig. 10.** Leather dyed with beetroot colorant. Left: with ultrasonic extract; right: with magnetic stirring extract.

**Fig. 11.** Influence of ultrasound, 80 W in natural beet dye exhaustion during the course of the leather dyeing process.

**Fig. 12.** Spent dye liquor from the dye bath. Left: with ultrasonic process; right: with magnetic stirring process.
general practice to use conventional metal mordents in dyeing with natural materials. Since the application here involves health sensitivity, metal mordents may pose toxicity, which is undesirable. Hence, research on suitable eco-friendly alternative mordents is being pursued in our laboratory for improving the wet rub and perspiration resistance as well.

3.12. Environmental benefits

Since the process involves natural material with non-toxic and nutritious nature, highly suitable for dyeing of fibrous substances such as leather for health sensitive applications. Earlier studies confirm that beet colorants pose no toxic effects [18] as well as biodegradable [19] and contain negligible amount of phenolics component [20]. Therefore, there is no environmental risk involved in spent dye liquor unlike in the case of azo dyes. Even the spent dye liquor can be recycled for the next operation and develop no unpleasant odours. In addition, the use of ultrasound in beetroot dyeing process offers significant improvements in exhaustion as an in-plant control measure to achieve near zero discharge concepts. Beet extract obtained can be spray dried to get beet colorant in powder form and solvent ethanol can be recovered by suitable solvent recovery system.

3.13. Economic benefits

There is a significant improvement in the yields of extract obtained due to the use of ultrasound, even for the higher amounts of beetroot (1–8 g) used for the same ultrasonic power of 80 W. This indicates scale-up viability of the process. The environmental factor and health concern involved in the dyeing application overweigh the cost factor as compared to toxic synthetic dyes.

4. Conclusions

The use of ultrasound is found to have significant improvement in the extraction efficiency of colorant obtained from beetroot as reported for the first time. Based on the experiments it has been found that 1:1 ethanol–water mixture with 80 W ultrasonic powers for 3 h extraction provided better yield and extraction efficiency. Pulse mode operation may be useful for reducing electrical energy consumption in the extraction process. Two-stage extraction process has been studied and found to be beneficial for improving the yield for higher amounts of beetroot. Significant 8% enhancement in % yield of colorant has been achieved with ultrasound, 80 W as compared to MS process using 1:1 ethanol–water. This technique can also be employed as an analytical method for extracting coloring matter from plant materials such as beetroot. The coloring ability of extracted beet dye has been found to be suitable for leather as well as paper dyeing. Ultrason is also found to be beneficial in natural dyeing of leather with improved rate of exhaustion. The mechanism for the enhancement in extraction with ultrasound may be due to rupturing of plant cell wall, release and improved transport of beet dye in to the external medium mediated through micro stirring and acoustic streaming effects induced by ultrasonic cavitation. Therefore, the present study clearly offers better extraction methodology from natural dye resources such as beetroot even dispensing with external heating and for the dyeing of fibrous substances such as leather. The dyeing with beet material could be essential for applications, which involve health sensitivity. Thereby, making eco-friendly non-toxic dyeing of fibrous substances a potential viable ‘Green chemistry’ option for dyeing industries in near future amidst growing environmental concern.

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Fig. 13. White sheet of paper dyed with beet dye. Left: with ultrasonic extract; right: with magnetic stirring extract.