



## Studies on the Dyeing Properties of Cotton and Antimicrobial Activity of Natural Colourant Extracted from *Beta vulgaris* (Beetroot)

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### Abstract

Natural colourant was extracted from beetroot (*Beta vulgaris*) with Soxhlet apparatus using water, ethanol, ethyl acetate and aqueous methanol (50%). Methanol (50%) gave a higher yield of dyestuff than the other solvent. The stability of the dye on pH and temperature was best found to be 4.5 and 50°C respectively. The purification and separation of the extract was done using TLC and micro column, the better R<sub>f</sub> values were found to be 0.47, 0.36 and 0.24 when methanol and hexane (4:1) were used as the solvent. FTIR was used to characterize the extract and it showed that active component in the dye was present. Analysis of the dye was done with UV/visible spectrophotometry at a 538 λ<sub>max</sub>. The cotton fabrics were dyed with crude extract and a mordant (ferrous sulphate and alum) under conventional dyeing techniques; pre-mordanting and meta-mordanting, adopting a well-known vat dyeing method. The dyed fabric possessed very good fastness to light, rub and press but fair wash fastness. Ferrous sulphate as a mordant gave a better % exhaustion on dyed cotton and the dyeing was best achieved with pre-mordanting techniques. The antimicrobial activity test showed that, the extract was active against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* but non-active against *Candida albican*.

### 1. Introduction

Natural dyes have been used extensively since ancient periods for the purpose of

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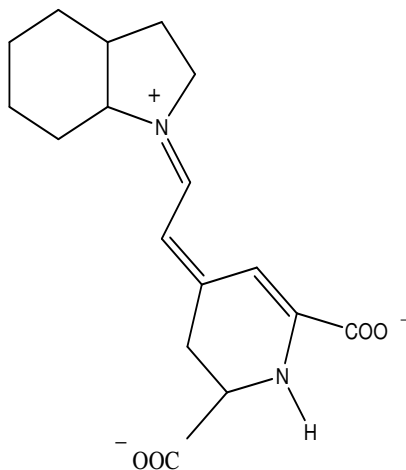
colouring textile material, until synthetic dyes were investigated and commercialized. The earliest written record of the use of natural dyes was found in China dated 2600 BC [1]. Due to, availability of synthetic dyes, superior fastness properties and its cost advantages most of textile manufacturers shifted towards use of synthetic dyes. Almost all the synthetic dyes are being synthesized from petrochemical sources through hazardous chemical processes poses threat towards its eco-friendliness [2]. Synthetic dyes which produce a wide variety of colours, sometimes cause skin allergy and other harms to human body, release undesirable and toxic chemicals during its synthesis [3].

On the other hand, natural dyes have better biodegradability and generally have higher compatibility with the environment. They are non-toxic, non-allergic to skin, non-carcinogenic, easily available and renewable [4, 5, 1, and 6]. There has been revival of the growing interest on the application of natural colourants in application of textile dying. Germany was the first to take initiative to put ban on numerous specific azo-dyes for their manufacturing and applications. Netherlands, India and some other countries also followed the ban [7]. However, Samanta and Agarwal [3] stated that colourants extracted naturally produce uncommon, soothing, and soft shades as compared to synthetic colourants but nevertheless there are few reasons why scientist and researchers are interested to study the potential of natural colourant; Wide viability of natural colourants and their huge potential, availability of experimental evidence for allergic and toxic effects of some synthetic dyes, and non-toxic and non-allergic effects of natural colourants, specialty colours and effects of natural dyes produced by craftsman and artisans for their exclusive technique and specialty work, availability of scientific information on chemical characterizations of different natural colourants, including their purification and extraction, availability of knowledge base and database on application of natural colourants on different textiles.



Beetroot

Beetroots (*Beta vulgaris*) are believed to originate along the coasts of the Mediterranean (sea beets) and were first cultivated for their edible leaves. The beetroot, commonly called the beet, is a biennial plant that produces seeds the second year of growth and is usually grown as an annual for the fleshy root and young leaves. The *Beta vulgaris* has three basic varieties: chard, grown specifically for its leaves; beets, grown for its bulbous root, with edible leaves (with varieties in white, yellow and red roots); and sugar beets, grown for making sugar from the long, thick root [8].



Betanin a betacyanin

Betalains are a class of red and yellow indole-derived pigments found in plants of the Caryophyllales, where they replace anthocyanin pigments. Betalains also occur in some higher order fungi [9]. They are most often noticeable in the petals of flowers, but may color the fruits, leaves, stems, and roots of plants that contain them. They include pigments such as those found in beets. The name “betalain” comes from the Latin name of the common beet (*Beta vulgaris*), from which betalains were first extracted. The deep red color of beets, bougainvillea, amaranth, and many cactuses results from the presence of betalain pigments [10]. Betalains have been found in fluorescent flowers [8]. Furthermore, plant physiologists are uncertain of the function that betalains serve in those plants which possess them, but there is some preliminary evidence that they may have fungicidal properties [11]. The most heavily studied betalain is betanin, also called beetroot red after the fact that it may be extracted from red beetroots [10]. Betanin is a glucoside, and hydrolyzes into the sugar glucose and betanidin [10]. It is used as a food coloring agent, and the color is sensitive to pH. The color and antioxidant capacity of

betanin and indicaxanthin (betaxanthin derived of L-proline) are affected by dielectric microwave heating [12]. Addition of 2,2,2-trifluoroethanol is reported to improve the hydrolytic stability of some betalains in aqueous solution [13]. The colour of red beetroot is due to betanin, which can be extracted easily from the vegetable. It makes up 95% of the red pigments in the extract. Betanin is a betacyanin a group of red dyes once thought to be flavonoids, like anthocyanins they are glycosides. However, their molecules contain nitrogen, they do not change colour reversibly when pH changes as anthocyanins do. Cotton is a vegetable fiber hence it originated from natural source. Botanically it belongs to the *malvaceae* family and its name *Gossypium* (*Gossypium* spp) [14]. Cotton is hair-like material, attached to the seed of certain plant of genus; *gossypium*. It looks a flat, twisted, ribbon-like material [15]. Cotton constitute cellulose 85-94%, oil and wax matter 0.5-0.7%, protein, pectose and colouring matter 4.5-5.0%, mineral matter 0.8-1.0%, moisture 0.8% [15]. Every textile material has the presence of tiny microscopic pores through which the dye molecules enters, and interact with the polymer matrix of the fiber. Cotton can be dyed with dyes of large molecules because the pores sizes are large. When the fiber absorbs water it swells, this facilitates the absorption of the dye by the fiber. Cotton can be dyed with large molecular weight dyes due to large surface area. Dyeing of cotton is faster at higher temperature, this is because the fiber swelling is higher at higher temperature than at lower temperature, and also mordanting increases the dye exhaustion by fixing the dye on to the fiber matrix [16].

## 2. Materials and Method

### 2.1. Materials

All chemicals and solvents used for this experiment were analytical-grade and were used without further purification. Ethanol (99.8%), methanol (50%), acetone (99.9%), n-hexane (97.0%), ethyl acetate (99.0%), HCl, sodium hydroxide (97%), acetic acid (99.8%) and sodium carbonate (97%). Mordants used were potassium aluminium sulfate ( $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ ), and ferrous sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) all were obtained from Sigma Alderich Germany.

### 2.2. Preparation of beetroot

The vegetable was collected fresh from farm, washed to remove dirt, the sample was peeled and cut into small pieces about 0.5-0.8 mm. The sample was dried under shade for

seven days. Then the sample was further dried in a desiccator. The dried samples were ground in a porcelain mortar in order to achieve high surface area for proper extraction [17].

### **2.3. Extraction process**

The extraction was carried out according to a similar procedure reported by Goodarzian and Ekrami [18] with some modification. 10 g of the sample was taken into a thimble and placed in a Soxhlet apparatus, 200 ml of solvent was added. The setup was then mounted on heating mantle and kept at 60°C for 24 hours. The system was cooled down to room temperature and concentrated using rotary evaporator. The obtained residue was dried overnight and finally a crude dried extract was obtained. The dye was extracted from beetroot using methanol, ethanol, water and ethyl acetate as a solvent of extraction, using the same method above and the yield was determined. 1ml of the extracts were dissolved in 100 ml of distilled water and analyzed using UV/Vis spectrophotometer.

### **2.4. Scouring of cotton fabrics**

Scouring of the fabrics was done by washing in a solution containing 0.5 g/l sodium carbonate and 2 g/l detergent at 50°C for 25 min, keeping the material to liquor ratio at 1:40. The scoured material was then rinsed and dried [19].

### **2.5. Mordanting**

The weighed cotton fabrics were treated with (ferrous sulfate and alum) salts for pre-mordanting and meta-mordanting. In pre-mordanting, fabrics were immersed in the mordant solution at 60°C for 30 min prior to dyeing. In meta-mordanting, fabrics were immersed in a dyeing bath containing both mordant and dyestuff for 120 min [20].

### **2.6. Dyeing method**

Cotton fabrics were dyed with betanin dye at concentration of 5 mg/ml and 50:1 liquor ratio. The dried scoured fabrics were introduced in to the dye bath and dyed at duration of (120 min) and at temperature (40-90°C). The dye bath was monitored and controlled at pH 4.5 with a pH meter, using dilute solutions of sodium carbonate and hydrochloric acid. The dyed samples were rinsed with cold water and finally dried [20].

### **2.7. Measurement of dye exhaustion by UV/Vis spectroscopy**

UV/Vis spectrophotometer was used to measure the exhaustion of the dyestuff. By

measuring the concentration of dye bath before and after the dyeing process, the percentage of exhaustion was estimated using the equation (1) [21].

$$\% \text{ exhaustion} = \frac{C1 - C2}{C1} \times 100. \quad (1)$$

## **2.8. Evaluation of colour fastness properties**

### **2.8.1. Fastness to light**

The light fastness properties of the dyed samples were assessed using the standard (B.S 1006) method. Xenon arc lamp was used which is an artificial light source representative of natural day light D65. Fabrics of measurement 7cmx12cm of cotton fabrics were exposed to Xenon arc lamp for 168h, at standard testing conditions using blue wool as standard reference fabric. The treated cotton fabrics were evaluated for colour fastness to light by comparing with grey scales [17].

### **2.8.2. Fastness to wash**

Wash fastness of the dyed fabrics was determined according to ISO 105 C06 method. Washing was done by preparing the soap solution containing 4g detergent and 1g sodium per borate solution of distilled water. Then pH was adjusted to 10.5 by the addition of 1g of sodium carbonate. The sample together with standard was washed for 30 min at 60°C with detergent solution having liquor ratio 50:1. After 30 min samples were removed, rinsed and dried. Afterwards the dyed samples were analyzed for colour change and staining with the 5 grades grey-white scale in D65 illuminate [22].

### **2.8.3. Fastness to rubbing**

A piece of the dyed fabric was rubbed on plane dry white cloth and also on a wet white cloth respectively. The staining of the white cloth was observed and assessed using grey scale [22].

## **2.9. Antimicrobial activity test**

### **2.9.1. Preparation of minimum inhibition concentration of the extract**

Minimum inhibitory concentrations of the extract were prepared by serial dilution using distilled water to obtain concentration of 4000 µg/ml, 2000 µg/ml, 1000 µg/ml and 50 µg/ml from the stock solution of 0.08 mg/ml or 8000 µg/ml. 0.5 ml was taken from the stock and replaced with the equal volume of distilled water.

### 2.9.2. Sensitivity testing of the extract

Screened and standardized inocula of each isolate were swabbed onto the surface area of the agar in a separate petri dishes and disc of the extract with standard antibiotics (gentamycin) and anti-fungal (ketoconazole) respectively. 0.01ml of the test concentration extract were pipetted and added in to the holes in the disc plate containing the agar and isolates. The plate were inverted and allowed to stand for 30 min for the extract to defuse in to the agar after which the plates were incubated aerobically at 35°C for 18 hr.

## 3. Result and Discussion

### 3.1. Solvent extraction

The betanin extract was obtained as brick red liquid using water, ethanol and (50%) methanol, while brownish yellow extract was obtained with ethyl acetate as a solvent of extraction. Aqueous methanol (50%) gave a higher yield of extract than the other solvent. It was observed that the amount of extracted colour increased with increasing polarity of the organic solvents, methanol (50%) was the most suitable solvent for extraction followed by water, ethanol and then ethyle acetate. The result is shown in Table 1.

**Table 1.** Amount of extracted betanin in percentage.

SOLVENT	% EXTRACTED BETANIN
Water	4.5
Ethanol	3.8
Methanol (50%)	5.3
Ethylacetate	3.3

### 3.2. TLC analysis

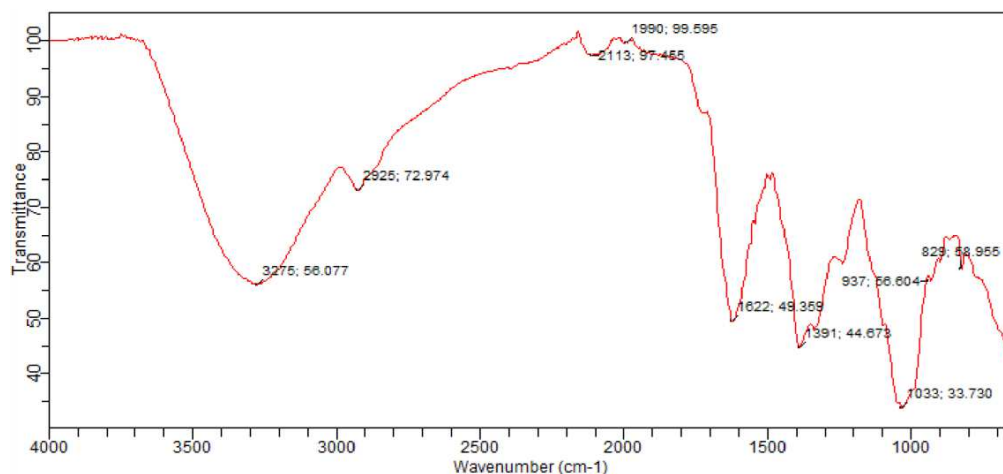
Different solvent combinations were tested using TLC plate for the separation of betanin dye. The better R<sub>f</sub> values were found to be 0.47, 0.36 and 0.24 when methanol and hexane (4:1) were used as the solvent. The R<sub>f</sub> values of the components were calculated, which are shown in Table 2. Hence the mixture of methanol and n-hexane in the ratio of 4:1 was suitable eluent for the separation of betanin in column chromatography.

**Table 2.** Rf values using different mobile phase.

TLC mobile phase	Ratio	Rf values
Et.acetate:meOH	6:1	0.47
n-hexane:meOH	4:1	0.24
n-hexane:Et.aceate	3:1	0.36

### 3.3. FT-IR spectroscopy

Figure 1 showed the FT-IR spectrum of the betanin extract. The peak at  $1048\text{ cm}^{-1}$  is assigned to C-O stretching vibration. The peak at  $1435\text{ cm}^{-1}$  corresponds to aromatic C-C stretching. The peak at  $2925\text{ cm}^{-1}$  corresponds to C-H stretching vibration. The broad peak at  $3275\text{ cm}^{-1}$  is assigned to the O-H stretching. The peak at  $1685\text{ cm}^{-1}$  is assigned to C=O vibrations of unsaturated ketones. For betanin dye, most of the active functional groups such as C=O (which are attributed to carbonyl) and O-H (corresponds to the hydroxyl group) are usually from carboxylic acid. The presence of carboxyl group contained in betanin promotes strong hydrogen bonding, this resulted in a large shift of the O-H bond to a lower frequency. The presence of COOH stretching vibration confirms the carboxyl group in betanin derivatives and is well matched with FT-IR absorption spectra [23].

**Figure 1.** FTIR spectrum of betanin extract.



### 3.4. Dye exhaustion

The result for percentage exhaustion of dyed cotton fabric is shown in Table 3. It has been observed that dyeing with betanin dye was best achieved by pre-mordanting, than simultaneous mordanting method and without mordant. From the result ferrous sulfate as mordant showed a better exhaustion than alum the highest exhaustion was 96% pre-mordanted using ferrous sulfate and least 22% without mordant.

**Table 3.** Maximum exhaustion of betanin dye by cotton fabric.

FABRIC DYED WITH BET	DYE EXHAUSTION (%)
Cotton pre-mordanted with ferrous sulfate	96
Cotton pre-mordanted with alum	80.6
Cotton meta-mordanted with ferrous sulfate	90
Cotton meta-mordanted with alum	74
Cotton without mordant	22

### 3.5. Light fastness properties

Fastness to light of the cotton fabric dyed with betanin extract showed magnificent result. Dyeing without mordant gave moderate to good result (5-6). Pre-mordanting dyeing with both alum and ferrous sulfate gave very good to excellent result (7-8). Meta-mordanting dyeing with alum gave moderate result (6-7) while meta-mordanting dyeing with ferrous sulphate showed very good results (7-8).

**Table 4.** Light fastness properties of dyed cotton fabric.

DYEING TECHNIQUE	LIGHT FASTNESS
Cotton pre-mordanted with ferrous sulfate	7-8
Cotton pre-mordanted with alum	7-8
Cotton meta-mordanted with ferrous sulfate	7-8
Cotton meta-mordanted with alum	6-7
Cotton without mordant	5-6

### 3.6. Wash fastness

The results for the wash fastness test for the dyed cotton fabrics according to ISO 105-C is presented in Table 5. Grade range from 1-5, where 1 represents very bad and 5 represents very good. The ratings for the colour change of unmordanted and meta-mordanted samples dyed with betanin were fair (2-3). While the ratings for the color change of pre-mordanted show good (3-4). The rating of change in stain of pre-mordanted with ferrous sulphate is bad (1-2), change in stain of pre-mordanted with alum, meta-mordanted with both alum and ferrous sulphate showed fair (2-3), while for the unmordanted showed poor (2).

**Table 5.** Washing fastness properties of dyed cotton fabric.

DYEING TECHNIQUE	LIGHT FASTNESS	
	Colour change	staining
Cotton pre-mordanted with ferrous sulfate	3-4	1-2
Cotton pre-mordanted with alum	3-4	2-3
Cotton meta-mordanted with ferrous sulfate	2-3	2-3
Cotton meta-mordanted with alum	2-3	2-3
Cotton without mordant	2-3	2

### 3.7. Rubbing fastness

Change in shade in case of dry rubbing of cotton fabrics dyed without mordant, pre-mordanted with alum, meta-mordanted with alum showed good result (3-4) whereas cotton pre-mordanted with ferrous sulphate and meta-mordanted with ferrous sulphate showed very good to excellence result (4-5). While change in shade in case of wet rubbing of cotton pre-mordanted with alum and meta-mordanted with ferrous sulphate gave very good to excellence results (4-5) and cotton pre-mordanted with ferrous sulphate, meta-mordanted with alum and cotton without mordant showed good results (3-4). Generally the dyed fabrics showed a remarkable rubbing fastness as presented in Table 6.

**Table 6.** Rubbing fastness properties of dyed cotton fabric.

DYEING TECHNIQUE	RUBBING FASTNESS	
	Dry rubbing	Wet rubbing
Cotton pre-mordanted with ferrous sulfate	4-5	3-4
Cotton pre-mordanted with alum	3-4	4-5
Cotton meta-mordanted with ferrous sulfate	4-5	4-5
Cotton meta-mordanted with alum	3-4	3-4
Cotton without mordant	3-4	3-4

### 3.8. Antimicrobial activity test

Table 7 showed the inhibition zone by isolates in response to beetroot extract with standard gentamycin and ketoconazole disc. Aqueous extract of betanin were active against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* at concentration of 4000, 2000, 1000 and 500 µg/disc. Wide inhibition zone was observed against *Staphylococcus aureus* with 21 mm diameter of 4000 µg/disc, which signified that it has more sensitivity against the organism than the others. While least sensitivity was found in *Aspergillus niger* with 18 mm diameter of 4000 µg/disc, the betanin extract and standard gentamycin was active against the bacteria with clear inhibition zone. It was worthy that, no activity was found by *Candida albican* neither with the betanin extract nor with the standard gentamycin.

**Table 7.** Antimicrobial sensitivity test of beetroot extract against bacteria and fungi isolates at different concentration and standard control antibiotics used.

Test organism used	Concentration of extract (µg/ml)				Antibacterial	Antifungal
	4000	2000	1000	500	Control	Control
<i>Staphylococcus aureus</i>	21mm	19mm	16mm	12mm	26mm	-----
<i>Escherichia coli</i>	15mm	12mm	10mm	8mm	24mm	-----
<i>Aspergillus niger</i>	18mm	15mm	11mm	7mm	-----	-----
<i>Candida albican</i>	-----	-----	-----	-----	-----	-----

#### 4. Conclusion

The work showed that the aqueous organic solvent extraction of betanin dye from beetroot is possible. The four different solvents; water, alcohol, methanol and ethyl acetate were chosen for the extraction of betanin. Amongst them aqueous methanol (50%) was slightly better for the extraction due to its strong polar nature. Betanin dye was successfully separated and purified using TLC and column chromatography respectively. The result showed that betanin dye has affinity on cotton fabric. The dye percentage exhaustion also followed the same trend. It was observed that the use of mordant has significantly increased the light fastness of the dyed fabrics, but not much of the improvement in wash fastness. Pre-mordanting method was found to be more reproducible than the corresponding meta-mordanting method. However, the use of copper sulfate as a mordant showed better result than alum. The extract was found to possess a remarkable antimicrobial property.

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