Antibacterial efficiency of naturally occurring dyes and mordants

Taiyaba Nimra Ansari1 · Sanjeeda Iqbal1

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Abstract
The popularity of natural dyes is steadily increasing due to their properties such as soothing colors, non-carcinogenic & non-hazardous nature, and also being safe for the environment. Some natural dyes also have been reported to have antibacterial properties. This study was performed to explore the antibacterial efficiency of selected plant products that were used in the dyeing of fabric. Flowers of Butea monosperma and Tagetes erecta were used for the dye extraction. Banana pseudostem sap and Punica granatum rinds taken as biomordant. High-performance thin-layer chromatography was conducted for the identification of components of B. monosperma, T. erecta, Banana (Musa sp.) pseudostem sap and P. granatum rind. Dyeing of two types of cotton fabrics was performed at pre-optimized conditions. The antibacterial activities of dyed fabrics were evaluated qualitatively and quantitatively. Various phytochemical constituents in the dyeing plant materials already have antibacterial properties. Fabrics dyed with B. monosperma, T. erecta, Banana (Musa sp.) pseudostem sap and P. granatum rind showed high resistance towards Escherichia coli and Staphylococcus aureus.

Keywords Dye · Biomordant · Antibacterial · HPTLC

Introduction
Natural dyes are one of the most important natural products obtained from plants, animals, minerals, and micro-organisms. These dyes have been used to color fabric, food, cosmetic products, and decorative arts since ancient times. After 1856 with the discovery of synthetic dyes, the popularity of natural dyes has diminished (Gregory 2003; Chavan 2011). Textile industries are attracted to synthetic dyes which have low cost, fast colors and easy in application on textiles. Wide use of synthetic dyes for a long time resulted in carcinogenic and mutagenic effects, with their non-biodegradable nature (Kiumarsi et al. 2017). With rise in awareness of global warming, emissions and ecosystem health, green products are increasingly in demand (Sagnella et al. 2014; Sagnella et al. 2015). In this context, people are also taking interest in healthy and safe clothing. Cotton is one of the most common fabrics for apparel all over the world. Cotton is produced naturally and is a good substrate to grow microorganisms (Fu et al. 2011; Zhou and Kan 2015). As its hydrophilic nature is, cotton easily provides suitable conditions, such as humidity, adequate temperature, and nutrition, for the growth of microorganisms, which generate an unpleasant odor, stains, and discoloration of the fabric (Broadbent 2001). Therefore, it becomes necessary to apply an antibacterial finishing on cotton fabrics. Any substance which is used to kill or inhibit the growth of microorganisms is called an antimicrobial agent (Burnett-Boothroyd and McCarthy 2011). Antibacterial finishes are generally applied by using Spun-in additives, Padding, Spraying, Microencapsulation, Polymer modification, etc. (Billah 2018). Many natural dyes have resistance to bacterial growth (Mirjalili and Abbasipour 2013; Mishra et al. 2017). Therefore, in this research work dyeing of cotton fabrics is done with natural dyes and mordants to obtain safe clothing.

Flowers of Butea monosperma and Marigold were taken as dye sources. Pomegranate rind and Banana pseudostem sap were taken as mordants. Most of the natural dyes have a very low affinity towards fabric, so a mordant (fixing agent) is required to fix dyes on fabric (Singh and Bharati, 2014). Chromium, copper, iron, tin and tannic acid, etc. are metal salts used in synthetic dyes, and are extremely toxic to human and ecological health (Velusamy et al. 2021). Only a small amount of these metal salts get fixed on the fabric and the rest is discharged as effluent which leads to
the contamination of land and water resources (Saxena and Raja 2014). In this study, Pomegranate rind and Banana pseudostem sap are used as mordants which are generally considered as waste. Here is an attempt to utilize the waste materials in the dyeing process.

**Objectives**

- Evaluation of natural mordant qualities.
- Phyto-chemicals screening of dyes & mordants.
- Enumeration of antibacterial efficiency of dyed fabrics.

**Material and Methods**

**Selection and collection of dye**

Flame of forest (*Butea monosperma*) is a deciduous tree native to tropical and subtropical parts of the Indian subcontinent. The flower is long and bright orange. Flowers were collected between March and April and were plucked directly from plants. Petals were separated from the rest of the flowers and preserved. Marigold (*Tagetes erecta*) is an annual or perennial herbaceous plant. Petals were dried at room temperature and stored in airtight conditions (Fig. 1).

**Selection and collection of mordants**

1. Pomegranate (*Punica granatum*) is a deciduous shrub. Pomegranate fruit has anti-oxidant, anti-viral and anti-tumor properties. Therefore, it is used in large quantities by people. A Pomegranate is a sweet, tart fruit with a thick red rind. The skin is not edible and discarded, and has been used in present research work to find out its qualities.

2. Banana (*Musa* sp.) plant is a larger herbaceous flowering plant. The Banana plant dies after fruiting. Another sucker replaces it and the whole plant body such as leaves and pseudostem is thrown away as waste. Pseudostem contains liquid material in the leaf sheath.

In this concern both waste materials i.e. pomegranate rind and Banana pseudostem sap are taken as mordants (fixing agents) for dyeing (Fig. 2).

**Dyeing process**

**Fabric selection**

Two different types of cotton fabric were taken i.e. handloom cotton (Khadi) as fabric 1 and power-loom cotton as fabric 2 for this study. Khadi was purchased from Khadi Bhandar (Indore Khadi Sangh) Rajwada, Indore and fine cotton fabric procured from G. Sachanand Kala mandir, Hukumchand Marg, Indore (Madhya Pradesh).

**Dye extraction**

Dried powders of dyes were taken for the preparation of aqueous extraction of dye. Fifteen gram of dye powder (15% oven weight of fabric) was weighed and poured in a beaker and soaked in 100 ml water for 15 min at room temperature. Dye pot (beaker) was kept at high temperature (95–100 °C) for boiling and then the temperature was reduced to 60 °C and maintained for 1 h (Win and Swe 2008). The extraction liquor was then filtered to obtain an aqueous solution of dye. The temperature and time of extraction were selected based on reviewed literature and experimentation.

**Biomordanting**

Banana pseudostem was diced to small pieces and sap was extracted with the help of grinder and stored in air-tight bottles for further use. Dried Pomegranate rind was ground and soaked in water for 15 min then the extraction process was done for about an hour in the water bath at 80 °C. The extract was collected for mordanting (Kumaresan 2011).

**Dyeing**

Dyeing of fabric was performed under optimized conditions acquired through experiments. Fabric dyeing depends on the

![Fig. 1 Natural dyes: Flowers of *Butea monosperma* (a), *Tagetes erecta* (b)](image)

![Fig. 2 Natural Mordant: Rinds of *Punica granatum* (c), Pseudostem of *Musa* sp (d)](image)
concentration of mordants, pH, temperature and time. 15% concentration of dye was applied with both the mordants. However, different concentrations of mordants on cotton fabrics showed various color strengths. Concentrations of mordants which exhibited maximum color strength were taken for dyeing.

Sample selection for antibacterial activity study

Based on the color strength of both fabrics in each dye and mordant combination, 08 samples were selected to assess the antibacterial activity. Following are the concentrations of mordants that were selected for dyeing (Table 1).

HPTLC analysis

High-performance thin-layer chromatography was performed to identify the content of natural dyes obtained from flowers of Butea monosperma, Tagetes erecta and mordants such as dried powder of Punica granatum rinds and Musa sp (banana) pseudostem sap (Samanta and Konar 2011). HPTLC analysis was done at Anchrom Enterprises (I) Pvt. Limited by using HPTLC system (CAMAG, Switzerland). Predevelopment of stationary phase HPTLC silica gel 60F254 (Merck, Germany) was carried out by analytical grade methanol. HPTLC plate was kept in a pre-saturated twin trough chamber (10 × 20 cm) for the development of samples. Following standardized developing solvent system (mobile phase) and derivatization were applied for the analysis (Table 2).

After development, scanning was executed in the photodocumentation chamber (CAMAG REPROSTAR 3) and images were captured in White light, UV 254 nm and UV 366 nm. The results were examined by using HPTLC Server LABSERVER, VERSION 2.5.18262.1 software.

Determination of Rf values

The identification of the separated compounds is usually based on their reaction with suitable reagents and their position in the chromatogram.

The Rf value is constant for a given compound under standard conditions.

\[
\text{Relative front (Rf)} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}
\]

Antimicrobial activity assessment of dyed fabric samples

Antimicrobial activity was evaluated by both qualitative and quantitative test methods. The undyed fabric was taken as a control for the study.

Qualitative assessment by agar diffusion method (SN 195920-1992)

This method was used to find out the antimicrobial efficacy of the treated fabrics qualitatively according to the Swiss standard-SN195920. For this test, a bacteriostatic agar medium was used as a growth medium for evaluation. Non-sterile dyed samples of 1X1 cm and square in shape were taken as test specimens. Undyed fabric samples were used as control. The potato dextrose agar (PDA) was prepared and sterilized in an autoclave. In the laminar airflow chamber, PDA was added to a sterilized petri dish and allowed to harden for 30 min.

| Table 1 Selected concentration of mordant for each fabric |
|----------------------------------------------------------|
| Combination of dye and mordant                           | Concentration of Mordant (gm/100 ml) | Fabric 1 | Fabric 2 |
| Dye           | Mordant                |                           |     |       |
| Butea monosperma | Banana pseudostem sap | 25 | 20 |
|               | Pomegranate            | 25 | 30 |
| Marigold      | Banana pseudostem sap | 30 | 20 |
|               | Pomegranate            | 25 | 30 |

| Table 2 Mobile phase and derivative for class of compounds |
|------------------------------------------------------------|
| Class of compound | Mobile Phase | Derivative                                |
|-------------------|--------------|-------------------------------------------|
| Alkaloids         | Toluene: ethyl acetate: methanol: ammonia 25% (30:30:15:1) v/v/v/v | Dragendorff’s solution |
| Anthraquinones    | Ethyl acetate: methanol: water (8:2:0.1) v/v/v | Alcoholic KOH |
| Bitter principles | Ethyl acetate: methanol: water (8:2:0.1) v/v/v | vanillin sulphuric acid |
| Essential oils    | Toluene: ethyl acetate (9.3: 0.7) | vanillin sulphuric acid |
| Saponins          | Chloroform: acetic acid: methanol: water (6.4:3:2: 1.2: 0.8) | Anisaldehyde sulphuric acid |
| Steroids          | n-butanol: methanol: water (3:1:1) | Anisaldehyde sulphuric acid |
| Sterols           | Chloroform: ethyl acetate (4:6) | Anisaldehyde sulphuric acid |
| Triterpenoids     | n-hexane: ethyl acetate (1:1) | Anisaldehyde sulphuric acid |
| Flavonoids        | Ethyl acetate: formic acid: glacial acetic acid: water (10:0.5:0.5:1.3) | Natural product reagent |
The gram-negative *Escherichia coli* MTCC (Microbial Type Culture Collection and Gene Bank) 77 and gram-positive *Staphylococcus aureus* MTCC 96 culture (24 h incubated) of the test organism were then evenly spread on separate agar plates using a cotton swab. In this experiment, the above two organisms were taken as representative bacteria for evaluation. The dyed and control fabric pieces (swatches) were placed on the solid agar inoculated with different bacterial cultures. The petri dish was then incubated at 37 °C for 24 h in an incubator. At the end of the incubation time, the test dish was observed. The evaluation was made for the appearance of the zone of inhibition around the fabric sample (swatch) measured in mm (millimeter) with this formula:

\[ W = \frac{T - D}{2} \]

where \( W \) = width of clear zone of inhibition (mm); \( T \) = total diameter of test specimen and clear zone (mm); \( D \) = diameter of test specimen (mm).

**Quantitative assessment by percentage reduction test (AATCC 100-2004)**

**AATCC test method 100-2004: antibacterial finishes of textile material**

Swatches in 4.8 ± 0.1 diameter were taken from the test fabric. The swatches are stacked in a 250 ml wide-mouth glass jar with a screw cap. 1 ml of *Escherichia coli* (MTCC 77) and *Staphylococcus aureus* (MTCC 96) broth poured onto the swatches separately. Two observations were taken for 0 (zero) contact time and 18–24 h at 37° ± 2°. Then the neutralizing solution was added and the jar was shaken vigorously for 1 min. Serial dilutions were prepared for plating of nutrient agar. Bacterial counts as the number of bacteria per sample was observed in each petri plate. Percent reduction of bacteria calculated in all plates by the following formula:

\[ R = \frac{100(B - A)}{B} \]

where \( R \) = % reduction; \( A \) = the number of bacteria recovered from the inoculated dyed (treated) test specimen swatches in the jar inoculated over the desired contact period; \( B \) = the number of bacteria recovered from the inoculated dyed (treated) test specimen swatches in the jar immediately after inoculation (at “0” contact time).

**Statistical analysis**

The data was analyzed by using Microsoft office Excel 2007 (v12.0). The data were expressed as mean ± standard deviation (Rao and Richard 2012).

**Results and discussion**

In the present study, the dyestuff obtained from flowers of *Butea* and Marigold was used for coloring of cotton fabrics at optimized dyeing conditions with Banana pseudostem sap and Pomegranate rind as mordants. The detection of class of compounds in both dyes and mordants was done with high-performance thin-layer chromatography (HPTLC). Inhibitory effects of naturally dyed fabric were evaluated against gram-positive and gram-negative bacteria. *S. aureus* and *E. coli* are representatives of gram-positive and gram-negative bacteria respectively.

**Biomordanting of fabrics**

The natural substances used for the fixation of dye are known as bio-mordants. Biomordanting was performed on two different types of cotton fabrics. Banana pseudostem sap and Pomegranate rind extract were taken for the same. Orange and yellow dyes were obtained from the flowers of *Butea* and Marigold after extractions. For biomordanting of fabric, concentrations of mordants were selected through a series of experimental trials. Constant dye concentration was carried out for both fabrics. A range of orange and yellow shades have been developed on cotton through optimized parameters and appropriate mordanting. Banana pseudostem sap itself is pale yellow in colour which does not show any dominance on dyed fabrics whereas, Pomegranate rind extraction was dusty in color which showed a slight dusty effect on dyed fabric.

**HPTLC analysis of dyes and mordants**

Identification of a major class of the compounds in the dyes and mordants was done with the help of HPTLC Chromatography. The details of the detection of compounds are reported in Table 3.

HPTLC Chromatograms of *Butea monosperma*, Marigold, Pomegranate and Banana pseudostem sap are represented in Fig. 3.

Through comparison of HPTLC chromatogram of the samples with the chromatogram of standards of Alkaloids, Anthraquinones, Bitter Principles, Flavonoids, Saponins, Steroids, Sterols and Triterpenoids were detected. These compounds play an important role to provide special features to the dyes and mordants. Alkaloids are the important secondary metabolites that were initially discovered and used as early as 4000 years ago and are well recognized for their rich therapeutic potential (Amirkia and Heinrich 2014; Hussain and El-Ansary 2018). Alkaloids have anti-proliferative, antibacterial, antioxidant...
| Class of compound | Name of the sample | Track no | No. of bands | Rf (Relative front) | Color          |
|-------------------|-------------------|----------|--------------|---------------------|----------------|
| **Alkaloids**     | **Butea**         | 2        | 1            | 0.42                | Orange         |
|                   | Marigold          | 4        | –            | –                   | –              |
|                   | Pomegranate rind  | 6        | –            | –                   | –              |
|                   | Banana pseudostem sap | 8  | 1            | 0.42                | Orange         |
| **Anthraquinones**| **Butea**         | 2        | 2            | 0.6                 | Yellow         |
|                   |                   |          |              | 0.69                | Blue           |
|                   | Marigold          | 4        | 1            | 0.82                | Blue           |
|                   | Pomegranate rind  | 6        | –            | –                   | –              |
|                   | Banana pseudostem sap | 8  | 1            | 0.69                | Blue           |
| **Bitter Principles** | **Butea**       | 2        | 7            | 0.15                | Brown yellow   |
|                   |                   |          |              | 0.23                | Yellow         |
|                   |                   |          |              | 0.29                | Orange         |
|                   |                   |          |              | 0.49                | Yellow         |
|                   |                   |          |              | 0.54                | Yellow         |
|                   |                   |          |              | 0.81                | Violet         |
|                   |                   |          |              | 0.9                 | Violet         |
|                   | Marigold          | 4        | 6            | 0.15                | Brown          |
|                   |                   |          |              | 0.45                | Red Brown      |
|                   |                   |          |              | 0.55                | Red Brown      |
|                   |                   |          |              | 0.68                | Red            |
|                   |                   |          |              | 0.82                | Purple         |
|                   |                   |          |              | 0.89                | Purple         |
|                   | Pomegranate rind  | 5        | 1            | 0.12                | Brown          |
|                   |                   | 6        | 2            | 0.56                | Violet         |
|                   |                   |          |              | 0.81                | Purple         |
|                   | Banana pseudostem sap | 8  | 2            | 0.14                | Light yellow   |
|                   |                   |          |              | 0.82                | Violet         |
| **Essential oils**| **Butea**         | –        | –            | –                   | –              |
|                   | Marigold          | 4        | 1            | 0.12                | Blue           |
|                   | Pomegranate rind  | 6        | 1            | 0.09                | Blue           |
|                   | Banana pseudostem sap | –  | –            | –                   | –              |
| **Flavonoids**    | **Butea**         | 2        | 4            | 0.28                | Aqua green     |
|                   |                   |          |              | 0.32                | light aqua green |
|                   |                   |          |              | 0.73                | Blue           |
|                   |                   |          |              | 0.78                | Aqua green     |
|                   | Marigold          | 4        | 1            | 0.93                | Blue           |
|                   | Pomegranate rind  | –        | –            | –                   | –              |
|                   | Banana pseudostem sap | 8  | 1            | 0.96                | Blue           |
| **Saponins**      | **Butea**         | 2        | 4            | 0.27                | Green          |
|                   |                   |          |              | 0.67                | Blue           |
|                   |                   |          |              | 0.76                | Blue           |
|                   |                   |          |              | 0.97                | Blue           |
|                   | Marigold          | 4        | 2            | 0.76                | Blue           |
|                   |                   |          |              | 0.86                | Violet         |
|                   | Pomegranate rind  | 6        | 4            | 0.47                | Blue           |
|                   |                   |          |              | 0.59                | Blue           |
|                   |                   |          |              | 0.77                | light blue     |
|                   |                   |          |              | 0.85                | Violet         |
|                   | Banana pseudostem sap | 8  | 1            | 0.12                | Blue           |
potential which can be used for the development of drugs (Qui et al. 2014). Alkaloids are an assembly of naturally occurring chemical composites which are typically comprised of basic nitrogen atoms. They may also contain some neutral or weakly acidic compounds (Manske and Holmes 2014). Alkaloids are low molecular weight structures and form approximately 20% of plant-based secondary metabolites (Kaur and Arora 2015).

Anthraquinones impart color to plants and have been widely used in natural dyes. They are also used as a laxative and possess antifungal and antiviral characteristics. Anthraquinones as the name implies are photochemical compounds based on anthracene (three benzene rings joined together). Naturally occurring anthraquinones are a group of secondary metabolites structurally related to 1,10-dioxoanthracene and their glycosides.

The term bitter principle is usually used to indicate a group of natural products that have an extremely bitter taste and were traditionally used in liquid medications to stimulate appetite. Bitter principles are mainly of vegetative origin, essentially comprising C, H and O but rarely have N.

Essential oils are aromatic, volatile liquid obtained from plant material through steam distillation and named after the plant from which they are derived. These fragrant substances are chemically pure compounds that are volatile under normal conditions (Rios 2016). They also have a medicinal application due to their therapeutic properties as well as agro alimentary uses because of their antimicrobial and antioxidant effect (Anand et al. 2019).

Saponins inhibit anti-inflammatory and immune-boosting properties as well as antibacterial effects. In medicine, they are used in vaccine formulation to regulate immune function. Additionally, they act as antioxidants and scavenge oxidative stress. Saponins are naturally occurring bio-organic compounds having at least one glycoside linkage (C–O-sugar bond) at C-3 between a glycone and sugar chain.

A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration.

Sterols occur in the membranes of plants, animals, and microorganisms and are termed phytosterols, zoosterols, and myco-sterols, respectively (Gordon 2003). Sterols are monohydroxy alcohols with a four-ring core structure or steroid nucleus (Dupon 2005).

Flavonoids are a class of phenolic compounds widely distributed in plants. Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water (Trugo et al. 2003). They consist of two benzene rings connected by a short three-carbon chain. One of the carbons in this chain is connected to carbon in one of the benzene rings, either through an oxygen bridge or directly, which gives a third middle ring. They function as plant pigments and are responsible for the colors of many flowers and fruits. The

| Class of compound | Name of the sample | Track no | No. of bands | Rf (Relative front) | Color |
|-------------------|-------------------|----------|--------------|------------------|-------|
| Steroids          | *Butea*           | –        | –            | –                | –     |
|                   | Marigold          | 4        | 1            | 0.93             | Purple|
|                   | Pomegranate rind  | –        | –            | –                | –     |
| Sterols           | *Butea*           | 2        | 2            | 0.64             | light purple |
|                   | Marigold          | 4        | 4            | 0.5              | light purple |
|                   | Pomegranate rind  | 6        | 2            | 0.63             | Purple |
|                   | Banana pseudostem sap | 8  | 1            | 0.67             | Purple |
| Triterpenoids     | *Butea*           | 2        | 2            | 0.48             | Violet |
|                   | Marigold          | 4        | 3            | 0.66             | Purple |
|                   | Pomegranate rind  | 6        | 3            | 0.21             | Violet |
|                   | Banana pseudostem sap | 8  | 1            | 0.58             | Violet |
biological activities of flavonoids have been demonstrated as anti-inflammatory, antihepatotoxic, antitumor, antimicrobial, antiviral, enzyme inhibitors, and antioxidant, and as having central vascular effects. Flavonoids also possess antibacterial, antiviral, and anti-inflammatory effects (Mese-role 2002).
Triterpenoids (such as lucidenic acids and ganoderic acids) have been reported to exhibit various biological activities, such as antibacterial, antiviral, antitumor, antiosteoclastic differentiation activity, anti-HIV-1, hepatoprotection, antioxidation, antihypertension, cholesterol reduction, and anti-aggregation functions (Hsu and Yen 2014). Triterpenoids metabolism plays an important role in medicinal plant drug discovery, development, and clinical uses. Triterpenoids are compounds with a carbon skeleton based on six isoprene units which are derived biosynthetically from the acyclic C30 hydrocarbon, squalene. They have relatively complex cyclic structures, most being alcohols, aldehydes, or carboxylic acids (Ludwiczuk et al. 2017).

All the above compounds have been regulated to the class of secondary plant products and great interest to the pharmaceutical industries. All compounds represent antibacterial properties that help to inhibit the bacterial colonies on fabrics too.

Assessment of antibacterial activity of dyed fabrics

Antibacterial efficiency of natural dye was evaluated against gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*) and gram-negative bacteria *Escherichia coli* (*E. coli*) using Agar diffusion method and Percentage reduction test. Control (undyed) fabric showed no zone of inhibition. *Butea* + Banana pseudostem sap dyed fabric 1 showed maximum zone of inhibition of 11.00 ± 1.00 mm and 8.67 ± 1.53 mm with *E. coli* and *S. aureus* respectively. In fabric 2 Marigold + Banana pseudostem dye mordant combination showed maximum zone of inhibition of 7.67 ± 2.08 mm and 10.33 ± 2.52 mm against *E. coli* and *S. aureus* respectively. However, the Control (undyed) fabric showed no percent reduction. While the maximum percent reduction in quantitative assessment of fabric 1 Butea + Banana pseudostem sap exhibited 53 ± 4.58% and 48.67 ± 7.02% against *E. coli* and *S. aureus*. In fabric 2 Butea + Banana pseudostem sap

![Fig. 3 (continued)](image-url)
showed $50.33 \pm 2.52\%$ ($E.\ coli$) and Marigold + Banana pseudostem sap showed $54.33 \pm 2.52\%$ ($S.\ aureus$) bacterial resistance (Tables 4 and 5) (Figs. 4 and 5).

In general, Bacteria can easily grow on any substrate and cotton fabric is also a good source of food and nutrition. Two representative microorganisms are specified for this testing, $S.\ aureus$ and $E.\ coli$. The method is useful for testing against both Gram Positive ($S.\ aureus$) and Gram-Negative ($E.\ coli$) bacteria. Most types of $E.\ coli$ are non-dangerous and even help keep our digestive tract healthy. But some strains can cause diarrhea if we eat contaminated food or drink fouled water. While many of us associate $E.\ coli$ with food poisoning, we can also get pneumonia and urinary tract infections from different types of bacteria. 75–95% of urinary tract infections are caused by $E.\ coli$. Some versions of $E.\ coli$ make us sick by making a toxin called Shiga. This toxin damages the lining of our intestine. One especially bad strain, O157:H7, can make us very sick. It causes abdominal cramps, vomiting, and bloody diarrhea. It is the leading cause of acute kidney failure in children. It can also cause life-threatening symptoms such as: Adult kidney failure, Fever, Bleeding, etc. $S.\ aureus$ has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections as abscesses (boils), furuncles, and cellulitis (Sukumaran and Senanayake 2016). $S.\ aureus$ can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections.

Significant results were obtained by qualitative and quantitative assessment of antibacterial properties of naturally dyed fabrics 1&2.

In the case of quantitative antibacterial analysis percent reduction of both the bacterial strains were calculated in the combination of dye and mordant. The highest percent reduction of bacterial colonies was found in fabric 1 with the combination of $Butea$ and Banana pseudostem sap for $E.\ coli$ as well as $S.\ aureus$. Similar results were obtained for fabric 2. Another good combination of Marigold and Banana pseudostem sap has given better results of resistance for both fabrics and both the strains of bacteria. However, all the results of naturally dyed fabrics showed significant resistance to bacterial growth.

As far as qualitative assessment is concerned the zone of inhibition is exhibited by both fabric types with two bacterial strains and four combinations of dye and mordant. The biggest size of the zone of inhibition was observed in $Butea$ + Banana pseudostem sap dyed fabric 1 for $E.\ coli$ and Marigold + Banana pseudostem sap combination of fabric 2 for $S.\ aureus$ respectively. These results have shown excellent properties of naturally dyed fabrics for antibacterial effects.

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**Table 4 Qualitative antibacterial assessment of natural dyed fabric**

| Combination of dye and mordant | Zone of inhibition in mm (Fabric1) Mean ± SD | Zone of inhibition in mm (Fabric 2) Mean ± SD |
|-------------------------------|---------------------------------------------|---------------------------------------------|
|                               | $E.\ coli$                                   | $S.\ aureus$                                |
| Control (Undyed fabric)       | 0 (No zone seen)                             | 0 (No zone seen)                            |
| $Butea$ + Banana pseudostem sap | 11.00 ± 1.00                                 | 6.67 ± 1.53                                 |
| $Butea$ + Pomegranate          | 3.67 ± 1.53                                  | 2.33 ± 1.53                                 |
| Marigold + Banana pseudostem sap | 8.00 ± 1.73                                  | 5.33 ± 1.53                                 |
| Marigold + Pomegranate        | 1.33 ± 0.58                                  | 3.00 ± 1.00                                 |

*Note: Values are given in mean ± SD for three replicates*

**Table 5 Quantitative antibacterial assessment of natural dyed fabric**

| Combination of dye and mordant | Bacterial resistance in percent (Fabric 1) Mean ± SD | Bacterial resistance in percent (Fabric 2) Mean ± SD |
|-------------------------------|-----------------------------------------------------|-----------------------------------------------------|
|                               | $E.\ coli$                                           | $S.\ aureus$                                         |
| Control (Undyed fabric)       | No reduction                                         | No reduction                                         |
| $Butea$ + Banana pseudostem sap | 53.3 ± 4.58                                         | 48.67 ± 7.02                                        |
| $Butea$ + Pomegranate         | 39.33 ± 2.52                                        | 37.67 ± 3.21                                        |
| Marigold + Banana pseudostem sap | 50.00 ± 3.00                                       | 41.33 ± 2.31                                        |
| Marigold + Pomegranate        | 32.33 ± 3.21                                        | 40.33 ± 3.51                                        |

*Note: Values are given in mean ± SD for three replicates*
Where synthetic dyes and chemical mordants are hazardous to human life and the environment. The natural dyes and mordants give protection to users from harmful bacteria.

HPTLC analysis of dyes and mordants gave a class of phytochemicals generally present in all-natural dyeing materials. Antimicrobial properties of Alkaloids, Anthraquinones, Essential oils, Saponins, Flavonoids and Triterpenoids studied by many researchers are also present in dyes and mordants used in this investigation. All these six types of phytochemicals were analyzed in Butea and Banana pseudostem sap by chromatogram studies. Marigold showed 5 such phytochemicals and Pomegranate rinds analyzed the presence of only 3 chemicals having antibacterial properties. Both quantitative and qualitative assessments of naturally dyed fabric gave outstanding results, which might be shown due to the presence of these phytochemicals possessing...
natural antibacterial properties. Contribution of the higher number of these phytochemicals present in dye and mordant has shown the highest biocidal effects on fabrics as given in Tables 4 and 5. Therefore, excellent results of both the assessments shown by the combination of Butea + B.Sap and Marigold + B.Sap having the maximum number of such phytochemicals in them, which are responsible for antibacterial effects naturally. Such a type of natural dyeing gave an antibacterial finish to the fabric without any extra cost in an eco-friendly manner.

Thus, naturally dyed fabrics are a better choice to save the health of both human beings and the environment. Hence, the popularization of natural dyes, mordants and their use in dyeing and printing must be accelerated to preserve nature.

Conclusions

The research work has investigated a new innovation in the dyeing of cotton with natural dyes. The dyeability and fastness properties of dyed fabric were studied successfully. The phytochemical analysis is one of the best tools to know about the presence of compounds in plant material. HPTLC gives us a long list of phytochemicals present in dyes and mordants with antibacterial nature. The study of antimicrobial properties of any dyed sample is one of the very important parameters to know about the quality of dye and mordant that becomes a more valuable criterion when we work with natural dyes and mordants. In present investigations, the antimicrobial properties against E. coli and S. aureus revealed the significant results of biocidal qualities of natural dye and mordant combination. This research work was done with two representatives of the gram-positive and gram-negative bacteria groups. Thus, this also highlights the possibilities of antimicrobial activity of dyed fabric for many such broad spectra of bacteria present in nature which may cause harmful allergies and infections to humans. Synthetic biocides have some side effects on human skin and cause water pollution as well. Therefore, natural biocides are the best option to control microbial growth on fabric as well as reduce pollution in nature. The fabric dyed with colorant from Butea monosperma, Marigold, Banana pseudostem sap and Pomegranate rind extracts displayed excellent antibacterial activity against both test organisms used. Application of natural dye and mordant, which contains antimicrobial properties, will also reduce the extra expenses of synthetic biocides used by textile industries. In the present investigation, the dyed fabrics were found to be hygienic, healthy, non-toxic, antibacterial and environment friendly.

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Author contributions TNA performed the experiments. TNA and SI analyzed the data. TNA and SI wrote the manuscript. TNA and SI conceived the research. Both authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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