



## Antibacterial Efficacy of Natural Dye from *Melia composita* Leaves and Its Application in Sanitized and Protective Textiles

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### ABSTRACT

**Background:** In view of the increasing consciousness on higher level of hygiene and health safety, development of protective textiles through giving antimicrobial finish is in focus. Therefore, the present study was aimed to investigate the antibacterial activity of natural dye from *Melia composita* leaves and different types of dyed fabrics. **Methods:** Antibacterial efficacy of natural dye and dyed fabrics were evaluated against gram-positive bacteria, *Staphylococcus aureus*, *Streptococcus epidermidis* and *Bacillus cereus* and gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella flexneri* and *Proteus vulgaris* using agar well diffusion method. The minimum inhibitory concentration (MIC) was determined by serial dilution method. **Results:** The dye was found to have potent antibacterial against all the test bacteria at all the tested concentrations. Highest antibacterial activity against all the test bacteria was recorded with 50 mg/ml treatment, which was also higher as compared to positive control whereas minimum activity was found at 5mg/ml treatment. The MIC of natural dye against test bacteria was found in the range of 2.75-4.00 mg/ml. Dyed fabrics substrates (silk, wool and cotton) also showed significant antibacterial efficacy against the test bacteria. All dyed fabrics showed maximum reduction in *S. aureus* population and lowest in *E. coli*. Of the three dyed fabrics, silk exhibited the maximum reduction in all the test bacteria followed by wool and cotton. **Conclusion:** The study concluded that *M. composita* leaves can be a potential source of natural dye with remarkable antibacterial potency which can be applied in sanitized fabrics for medical applications and protective clothing.

**KEYWORDS:** *Melia composita*, Leaves, Natural Dye, Antibacterial activity, Protective textiles.

### INTRODUCTION

Natural dyes have been widely used in textile coloration since antiquity. At first, colorants were mainly extracted from minerals, insects and plants<sup>1</sup>. However, with advent of synthetic colourants during mid 19<sup>th</sup> century, a variety of competitive synthetic dyes captured the dye market on account of their low cost, larger colours range, greater fastness, ability to dye synthetic fibers and availability at large industrial scale<sup>2</sup>. Almost all these synthetic colorants being synthesized from petrochemical sources through hazardous chemical processes pose threat towards the environment and human body health<sup>3-5</sup>. Worldwide environmental consciousness coupled with increased awareness of environmental hazards of synthetic dyes has led to the revival of interest in natural dyes due to their non-polluting and non-toxic nature. Consequently, numerous researches in recent years have

focused on development of non toxic and eco-friendly natural dyes for textiles colouration<sup>6</sup>. Natural dyes are being preferred over synthetics owing to their eco-friendliness i.e. they do not create any environmental problems at the stage of production or use<sup>7</sup>. Furthermore, in addition to their dye-yielding characteristics, some of dye-yielding plants also possess medicinal value. Some natural dyes have intrinsic additional properties such as antibacterial, antifungal, moth proof, anti-allergy, anti-UV, etc.<sup>8-10</sup>

Textile materials are prone to microbial growth and multiplication resulting into discoloration, objectionable odour, dermal infection, product deterioration, allergic responses and other related ailment<sup>11</sup>. There is, therefore, an increased inclination across the world in functional textiles that can provide a higher level of hygiene, safety and health protection to people. In the last few decades, a range of textile products based on synthetic antimicrobial agents such as triclosan, metal and their salts, organometallics, phenols and quaternary ammonium compounds, have been developed and quite a few are also available commercially<sup>12</sup>. However, synthetic antimicrobial are a cause of con-

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cern due to the associated side effects, action on non-target microorganisms and water pollution. Hence, there is a great demand for antimicrobial textiles based on ecofriendly agents which not only help to reduce effectively the ailing effects associated to microbial growth on textile material but also comply with the statutory requirements imposed by regulating agencies. In recent past, considerable research has been done towards investigating the antibacterial properties of different plant derived natural dyes<sup>13-21</sup>. The present research was aimed to explore the *Melia composita* leaves as a source of antibacterial natural dye.

*Melia composita* Willd. Syn *M. dubia* Cav. commonly known as Bakayan is a fast growing tree occurring in tropical forests and commercially valued for its multifarious uses. It is an important timber species provide high-quality termite and fungus resistant timber<sup>22</sup>. In traditional system of medicine, the plant is regarded as remedy for fevers, stomach worm, eczema, leprosy, asthma, malaria, venereal diseases, cholelithiasis, acariasis and pain<sup>23,24</sup>. The plant has been investigated for various chemical constituents including alkaloids<sup>25</sup>, limonoids<sup>26</sup>. It is well known as a rich and valuable source of bioactive limonoids<sup>27</sup>. The plant is reported for wide range of biological activities like antiviral<sup>28</sup>, antimicrobial<sup>29</sup>, antifeedant activity<sup>30</sup>. Fruits of the plant are reported to have hypolipidemic<sup>31,32</sup> and antidiabetic<sup>33</sup> properties. In view of the research reports, it is clear that despite the therapeutic properties, *Melia composita* have not been investigated with reference to its potential as a source of antimicrobial natural dye. The study thus envisaged to investigate the antibacterial efficacy of natural dye extracted from leaves of *M. composita* as well as different kinds of textile fabrics dyed with the natural dye.

## MATERIALS AND METHODS

### Plant materials and fabrics

Fresh leaves of *Melia composita* were collected from one of the experimental stations of Forest Research Institute located at Kerry Village, Premnagar, Dehradun. The collected leaves were properly cleaned under running tap water and then allowed for air drying in shade. The air dried leaves were chopped into small pieces and coarsely ground (50 mesh) using electric grinder. Powdered leaves were preserved in sealed plastic containers for further studies. Silk, wool and cotton textile fabrics were purchased from an authorized outlet of KVIC, Dehradun. The fabric were washed with nonionic detergent (1% owf) for 30 min, rinsed and dried at room temperature. The scoured material was wetted in water for 30 min prior to dyeing or mordanting.

### Extraction of dye

Natural dye from *M. composita* leaves was extracted under pre-opti-

mized conditions of material to liquor (water) ratio (MLR), pH and time. Leaf powder was taken in a beaker and immersed in distilled water as per optimized MLR. The extraction was done for 70 min at the boiling temperature. Then, the solution temperature was allowed to cool at room temperature and was filtered with Whatman (No. 1) filter paper. The filtrate was distilled under reduced pressure and finally dried over dehydrating agent in vacuo that resulted in natural dye powder.

### Dyeing of Fabrics

All the fabric samples were cleansed thoroughly off starch and other impurities and rinsed with cold water and dried. Silk, wool and cotton fabrics were dyed with solution of natural dye extracted by methods mentioned above. At the end, the dyed samples were removed, rinsed under running tap water in order to remove non-absorbed dyes and then dried at ambient conditions. It is worth mentioning that all dyeing of all fabrics were performed under optimum conditions of MLR, pH, temperature and time acquired through experiments.

### Test solutions of natural dye

Test solutions of a series of concentrations viz., 5, 10, 20, 30 and 50 mg/ml were prepared by dissolving natural dye obtained from *M. composita* leaves in dimethyl sulfoxide (DMSO). All test solutions were kept in refrigerator at 4°C for future use.

### Bacterial strains

Antibacterial activity of natural dye obtained from *M. composita* leaves and the fabric dyed with the natural dye was tested against gram positive bacteria, *Staphylococcus aureus*, *Streptococcus epidermidis* and *Bacillus cereus* and gram negative bacteria, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella flexneri* and *Proteus vulgaris*. The pure bacterial cultures were maintained on nutrient agar medium and each bacterial culture was further maintained by subculturing on the same medium and was stored at 4°C before use in experiments.

### Preparation of media

The medium was prepared by dissolving Muller Hinton Agar Medium (HiMedia) in distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25 ml/plate) while still molten.

### Assessment of antibacterial activity of *M. composita* leaf dye

The antibacterial activity of natural dye was tested against seven bacterial isolates using agar well diffusion method<sup>34</sup>. The culture plates were inoculated with 0.1 ml of standardized inoculums ( $1.0 \times 10^5$  CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs.

Wells of 8 mm diameter were punched into MHA petriplates containing the bacterial inoculums with sterile cork borer. The wells were filled with test solutions of natural dye as prepared above with three replications for each treatment. Commercially available antibiotics viz., Ampicillin and Streptomycin discs (1.0 mg/disc each) were used as (+) control and Dimethyl sulfoxide (DMSO) as a (-) control. The plates thus prepared were left at room temperature for 15 minutes allowing the diffusion of the extract into the medium. After incubation for 24 hrs at 37°C, the plates were observed. Antibacterial activity was observed by an inhibition zone surrounding the well containing the natural dye. The zone of inhibition was measured and expressed in millimeters. Each experiment was repeated thrice and the mean and standard deviation of diameter of inhibition zones were calculated. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

#### Determination of MIC

The minimum inhibitory concentration (MIC) was determined through the broth dilution method.<sup>[38-39]</sup> The natural dye solution (1 mg/ml) and serial dilution of the solution with bacterial culture with respective inoculums were used. The lowest concentrations without visible growth were defined as MICs.

#### Evaluation of the antibacterial activity of dyed fabrics

The antibacterial activity of the dyed fabrics (silk wool and cotton) against pathogenic microorganisms, *S. aureus*, *S. epidermidis*, *B. cereus*, *E. coli*, *K. pneumonia*, *S. flexneri* and *P. vulgaris* was quantitatively evaluated according to AATCC 100-1999 test method<sup>35</sup>. Circular fabric swatches (5.00 ±0.1 cm) were placed in container and sterilized for 15 min at 121°C. An aliquot of 1000 µl bacterial suspensions (nutrient broth culture containing 1.0x10<sup>5</sup> CFU/ml) of each bacterium were added to the center of fabric swatches and incubated for 24 hr at 37±1°C. The fabric swatches were resuspended in dilution medium, vigorously shaken 1 min prior to the dilution. Ten fold serial dilutions were made to all samples. A fixed volume of each dilution (100 µl) was inoculated on nutrient agar plates and the plates were incubated at 37±1°C for 24 hr. Untreated circular fabric swatches of same dimension were taken as control. Viable colonies of bacteria on the agar plate were counted and the percentage of reduction in the number of bacteria was calculated using following formula:

$$R(\%) = A-B/A \times 100$$

Where R = Reduction of bacteria; A = No. of bacteria colonies in the control (untreated fabrics), and B = Number of bacteria colonies in the treated fabrics.

#### RESULTS AND DISCUSSION

In the present study, the inhibitory effect of natural dye extracted from the leaves of *M. composita* as well as textile fabrics including

silk, wool and cotton dyed with the natural dye were evaluated against altogether seven bacterial strains. The antibacterial activity of natural dye was determined using agar well diffusion method and quantitatively assessed on the basis of inhibition zone. The minimum inhibitory concentration (MIC) was determined by serial dilution method. Furthermore, antibacterial effectiveness of silk, wool and cotton fabrics dyed with the *M. composita* leaf dye were also assessed by standard method<sup>35</sup>.

#### Evaluation of antibacterial activity of natural dye

The antibacterial efficacy of *M. composita* leaf natural dye was evaluated according to their zone of inhibition against various pathogenic bacteria and the results (zone of inhibition) were compared with the activity of the standards, viz., Ampicillin and Streptomycin. The results as summarized in Table 1 revealed that the natural dye is a potent antibacterial against all the microorganisms studied at all the tested concentration.

**Table 1: Antibacterial activity of natural dye from *M. composita* leaves against test bacteria**

| Natural Dye Conc. (mg/ml) | Zone of Inhibition (in mm) |                       | Mean±SD          |                |
|---------------------------|----------------------------|-----------------------|------------------|----------------|
|                           | <i>S. aureus</i> ,         | <i>S. epidermidis</i> | <i>B. cereus</i> | <i>E. coli</i> |
| 5                         | 4.62±0.23                  | 3.29±0.53             | 4.59±0.33        | 3.19±0.41      |
| 10                        | 9.54±0.13                  | 8.25±0.31             | 8.64±0.25        | 6.85±0.35      |
| 20                        | 15.23±0.22                 | 13.63±0.26            | 15.71±0.23       | 12.63±0.23     |
| 30                        | 23.55±0.31                 | 21.87±0.33            | 25.63±0.25       | 20.29±0.31     |
| 50                        | 32.41±0.19                 | 29.23±0.31            | 31.81±0.41       | 25.53±0.53     |
| Ampicillin                | 25.03±0.13                 | 21.67±1.08            | 21.33±0.41       | 23.46±0.71     |
| Streptomycin              | 29.25±0.31                 | 30.93±0.19            | 31.81±0.41       | 25.53±0.53     |
| DMSO                      | &                          | &                     | &                | &              |

| Natural Dye Conc. (mg/ml) | Zone of Inhibition (in mm) |                    | Mean±SD            |
|---------------------------|----------------------------|--------------------|--------------------|
|                           | <i>K. pneumonia</i>        | <i>S. flexneri</i> | <i>P. vulgaris</i> |
| 5                         | 3.16±0.45                  | 2.96±0.43          | 3.13±0.23          |
| 10                        | 7.51±0.43                  | 6.51±0.25          | 5.19±0.43          |
| 20                        | 14.53±0.13                 | 11.53±0.13         | 12.83±0.19         |
| 30                        | 18.36±0.33                 | 16.36±0.35         | 17.36±0.35         |
| 50                        | 23.51±0.19                 | 21.51±0.19         | 22.29±0.33         |
| Ampicillin                | 22.67±0.71                 | 24.25±0.82         | 20.00±0.07         |
| Streptomycin              | 23.51±0.19                 | 21.51±0.19         | 22.29±0.33         |
| DMSO                      | &                          | &                  | &                  |

It is evident from table1 that test solution of the natural dye at concentration of 50 gm/ml showed highest antibacterial activity against all the test bacteria. The activity at this concentration was found even better than the positive control. Maximum inhibition zone diameter 32.41±0.19 mm was obtained in *S. aureus*, followed by *B. cereus* and *S. epidermidis* with inhibition zone diameter 31.81±0.41 mm and 29.23±0.31 mm respectively. Treatment of the natural dye at 5 mg/ml concentration however, showed restrained and minimum activity against all the test bacteria (Fig. 1).

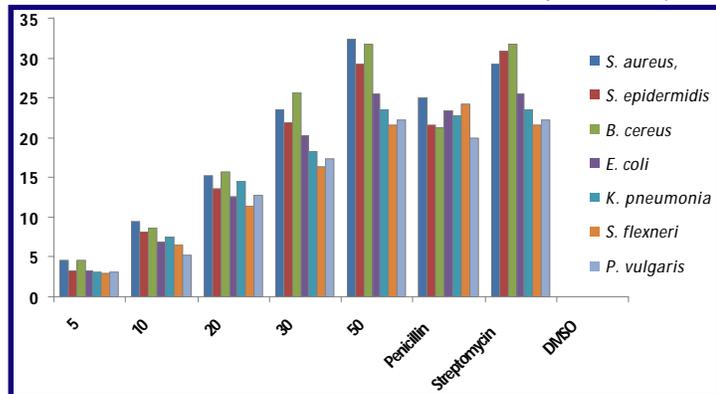


Fig. 1: Antibacterial activity of *M. composita* leaf natural dye against test bacteria

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is the lowest concentration able to inhibit any visible bacterial population. The MICs of *M. composita* leaf natural dye against test bacteria *S. aureus*, *S. epidermidis*, *B. cereus*, *E. coli*, *K. pneumonia*, *S. flexneri* and *P. vulgaris* were recorded as 2.75, 3.75, 3.25, 3.50, 3.75, 4.00 and 3.50 mg/ml (Table 2).

Table 2: MIC of natural dye against tested bacteria

| Bacterial strains     | MIC of Natural Dye (mg/ml) |
|-----------------------|----------------------------|
| <i>S. aureus</i> ,    | 2.75                       |
| <i>S. epidermidis</i> | 4.00                       |
| <i>B. cereus</i>      | 3.25                       |
| <i>E. coli</i>        | 3.50                       |
| <i>K. pneumonia</i>   | 3.75                       |
| <i>S. flexneri</i>    | 4.00                       |
| <i>P. vulgaris</i>    | 3.50                       |

MICs are important to confirm resistance of microorganisms to an antimicrobial agent and regarded as measurement of the activity of an antimicrobial agent against an organism.

Evaluation of antibacterial activity of dyed fabrics

As antibacterial assessment of *M. composita* leaf dye exhibited good antimicrobial activities against the test bacteria, it was thought appropriate to study their antibacterial activities on dyed substrate (fabric). The silk, wool and cotton fabric samples dyed with the natural dyes were used and their antibacterial effectiveness against the test bacteria was measured as percentage reduction in bacterial population. Results of the study are presented in Table 3.

Table 3: Antibacterial activity (reduction %) of dyed fabrics against test bacteria

| Dyed Fabric Substrates | Reduction in bacterial population (%) |                       |                    |                |
|------------------------|---------------------------------------|-----------------------|--------------------|----------------|
|                        | <i>S. aureus</i> ,                    | <i>S. epidermidis</i> | <i>B. cereus</i>   | <i>E. coli</i> |
| Silk                   | 94.85                                 | 92.68                 | 94.35              | 85.10          |
| Wool                   | 92.56                                 | 90.54                 | 91.63              | 79.78          |
| Cotton                 | 90.69                                 | 89.76                 | 90.06              | 75.52          |
| Dyed Fabric Substrates | Reduction in bacterial population (%) |                       |                    |                |
|                        | <i>K. pneumonia</i>                   | <i>S. flexneri</i>    | <i>P. vulgaris</i> |                |
| Silk                   | 87.01                                 | 85.75                 | 90.21              |                |
| Wool                   | 82.63                                 | 81.61                 | 85.95              |                |
| Cotton                 | 80.23                                 | 80.23                 | 82.55              |                |

It is evident from the results given in table 3 that all the three types of fabrics (silk, wool and cotton) dyed with *M. composita* leaf natural dye has significant antibacterial efficacy. The highest reduction was seen in the population of *S. aureus* whereas lowest in *E. coli* for three types of dyed fabrics. Silk fabrics dyed with natural dye exhibited the maximum reduction in population of all the seven test bacteria followed by dyed wool and cotton fabrics as visualized from Fig 2. Cotton fabrics dyed with the natural dye showed the minimum population reduction for all the test bacteria.

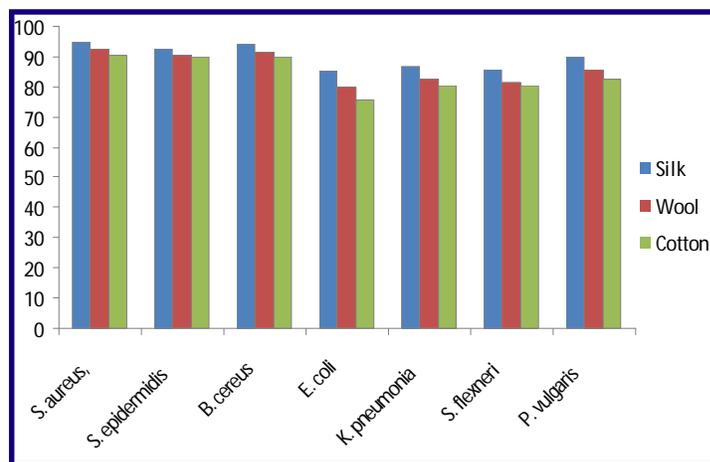


Fig. 2: Antibacterial activity (reduction %) of dyed fabrics against test bacteria

Many natural dyes obtained from various plants are known to have antimicrobial properties. *Lawsonia inermis* (Henna) a well known colorant for thousands of years is found to have antibacterial and antifungal properties<sup>36</sup>. The yellow pigment curcumin of Turmeric (*Curcuma longa*) is reported to have antiviral, antifungal, antibacterial effects and strong antiseptic potency<sup>37</sup>. Juglone from walnut and lapachol from alkanet are reported to exhibit antibacterial and antifungal activity<sup>37</sup>. *Punica granatum* (pomegranate) dye and many other common natural dyes are reported as potent antimicrobial agents owing to the presence of a large amount of tannins<sup>38,39</sup>.

Further, it is evident from results of antibacterial activity of *M. composita* leaf natural dye (Table 1) and dyed fabrics (Table 3) natural dyes had greater antibacterial activities towards the gram-positive bacteria (*S. aureus*, *S. epidermidis*, *B. cereus*) as compared to gram-negative test bacteria (*E. coli*, *K. pneumonia*, *S. flexneri* and *P. vulgaris*). It is reported that the cell wall structure of the gram-negative bacteria is constructed essentially with LPS that avoids the accumulation of the antimicrobial agents on the cell membrane<sup>40-42</sup>. The reports have confirmed the results of the present study.

CONCLUSION

Natural dyes derived from plant sources have long been known to

possess medicinal properties and consequently it seemed logical to explore their antibacterial activity considering the increasing interest and demand of functional textiles for protective clothing and clinical applications. In the current study, the antibacterial potency of natural dye obtained from leaves of *Melia composita* and different types of textile fabrics impregnated with the dye were evaluated. The results obtained showed that *M. composita* leaf dye have significant antibacterial activity. The study led to the conclusion that leaves of *Melia composita* can be a potential source of ecofriendly natural dye with remarkable antibacterial potency and the textile materials dyed with this natural dye can be very useful in developing sanitized fabrics for medical applications and protective clothing to protect users against common infections.

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