Research Article

Azadirachta indica Mediated Bioactive Lyocell Yarn: Chemical and Colour Characterization

B. H. Patel, K. U. Desai, and P. K. Jha

Department of Textile Chemistry, Faculty of Technology and Engineering, The M. S. University of Baroda, Vadodara, Gujarat 390 001, India

Correspondence should be addressed to B. H. Patel; kemchhobh@yahoo.com

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The study deals with preparing aesthetic textiles using methanolic extract of Azadirachta indica leaves. The extract with metallic and natural mordents was utilized to create various shades on lyocell yarn using exhaust technique of dyeing. Aesthetic values of dyed yarns were analyzed in terms of colourimetric parameters, that is, CIE $L^* a^* b^*$ and colour fastness. The attachment of Azadirachta indica compounds has been confirmed by using infrared spectroscopy (IR) analysis. The dyed samples exhibit moderate to good fastness properties. The study showed that lyocell yarn treated at 15% (owf) methanolic extract of Azadirachta indica leaves can be utilized as effective bioactive textiles. Azadirachta indica is an alternative to synthetic antimicrobial agents. This bioactive yarn can be used in fashion as well as in medicinal industry.

1. Introduction

Lyocell is regenerated fiber, manufactured by eco-friendly route [1]. Cellulose in the fiber has a high degree of orientation and the cellulose crystals are highly parallel in longitudinal direction of fibre. The fiber is soft to feel and highly absorbent and exhibits high strength in dry and wet conditions. Lyocell as staple fiber are used in clothes such as sportswear, casual wear, and towels. Filament fibers are used in items that have a silkier appearance such as women’s clothing and men’s dress shirts. Lyocell can be blended with a variety of other fibers such as silk, cotton, rayon, polyester, linen, nylon, and wool. Other than appeal lyocell is also used as industrial textiles, namely, in conveyor belts, specialty papers, and medical dressings [2].

Generally lyocell can be dyed with reactive class of dyes but it can be dyed with any class of dyestuff suitable for other cellulose [3]. Reactive dyes are criticized for the excess use of salt and alkali which may contribute in higher effluent and ultimately harm the environment. Therefore in the search of safe alternatives to dye lyocell, in the present study we have selected a dye extracted from Azadirachta indica leaves. Earlier we have applied Azadirachta indica leaves extract on nylon and Lycra textiles [4, 5].

This paper reports the results of the colouration of lyocell yarn by using the water and methanolic extracts from the leaves of Azadirachta indica. Azadirachta indica commonly known as “Neem” belongs to Meliaceae botanical family and is being abundantly available in nature. The principal constituents of these leaves include protein (71%), carbohydrates (22.9%), minerals, calcium, phosphorus, vitamin C, and carotene [6, 7]. An extract from the leaves can be prepared as an alcoholic tincture or as tea and this alcohol extract has a dark green colour and water extract has brown colour [8]. The chemical composition of the extract contains glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine and cystine like amino acids, and several fatty acids (dodecanoic, tetradecanoic, elcosanic, etc.). But physically the Azadirachta indica leaves have a pleasant odor and plant pigments like chlorophyll mainly responsible for green colour. The Azadirachta indica extract is reportedly officious against a variety of skin diseases, septic sources, and infected burns. The most important quality of these compounds is that they are less toxic to warm blooded animals like human [9]. Thus, considering its less toxicity and effectiveness against microorganism, it is expected to be one of the safest and most effective colourant cum antimicrobial agent for textiles.
Looking in the huge potential of bioactive textiles [4, 10–14] in this work an attempt has been made to develop bioactive lyocell yarn using a natural bioactive agent that is *Azadirachta indica* leaf extract. The aim of the project is to standardize and optimize the application process for water and methanolic extracts, in terms of colour strength and colour hue values. The chemical composition of *Azadirachta indica* leaf extract treated lyocell yarn has been characterized by FTIR spectroscopic analysis technique. To prepare lyocell yarn with aesthetic and medicinal properties, an attempt has also been made to evaluate the microbial efficacy of the yarn using bromophenol Blue and AATCC 100 methods.

2. Materials and Methods

2.1. Materials. Lyocell yarn and fabric, supplied by Birla Cellulose, Bharuch, Gujarat, (22ne - Denier) were used for the study. *Azadirachta indica* leaves were used as raw material to extract colourants. Two metallic mordants, that is, aluminum sulphate and ferrous sulphate, and two natural mordants, namely, *Harad* (*Terminalia Chebula*) and *Tamarind Kernel Powder* (TKP), were used without any purification.

2.2. Methods

2.2.1. Extraction of *Azadirachta indica* Dye. Extraction of dye from *Azadirachta indica* leaves was carried out by two ways: (i) water extraction and (ii) methanolic extraction. The leaves were dried at 110°C for short time, so that the volatile contents and green colour of leaves are not lost. These dried leaves were ground and converted into fine powder. This powder was further used for water and methanolic extractions.

For water extraction, 10 gm of obtained powder was added to 100 mL of water. The mixture was boiled for 2 hrs, till a brown coloured solution was obtained and filtered through Whatman filter paper number 1. Finally the total volume of extract was increased to 100 mL with distilled water. This solution was used as stock solution of 1:100 strength. In the case of methanolic extraction 10 gms of obtained powder was extracted in 100 mL of methanol at 50°C for 1 hr in Soxhlet apparatus. The extract was cooled and filtered through Whatman filter paper number 1. This extract was used as stock solution of 1:100 strength.

2.2.2. Dyeing with Water and Methanolic Extracts. Lyocell-hank was dyed with optimized dyeing conditions, that is, pH 8.5 and temperature 98 ± 2°C for 60 min in laboratory constant temperature shaking water bath, keeping material-to-liquor ratio of 1:50. Different concentrations, namely, 5, 10, and 15% (owf) of water and methanolic extracts of *Azadirachta indica* leaves, were added to the dyebath.

Uniform dyeing depends on various parameters such as pH, temperature, concentration of auxiliaries, and time of dyeing. To optimize these parameters, various trials of dyeing were conducted with water and methanolic extracts on lyocell. Hank of 1gm lyocell was prepared; these hanks were subjected to five different baths having different dyeing parameters. The optimum condition for dyeing was selected on the bases of higher colour strength (K/S) value.

Optimization of Dyebath pH. In order to optimize pH of the dyebath, dyeing was performed in absence of mordants with 10% (owf) dye concentration by exhaust technique. Acetic acid and sodium hydroxide with buffer tablets were utilized for maintaining the acidic and alkaline medium, respectively. Fixation of the dye was carried out in laboratory constant temperature shaking water bath at 98 ± 2°C for 45 min. The final result in terms of higher colour strength value was selected as optimized pH of dye liquor.

Optimum Temperature for Dyeing. To optimize the temperature for dyeing, experiments were conducted at different temperatures ranging from room temperature to boil, that is, 40 (R.T.), 50, 70, 90, and 98 ± 2°C at optimized pH. The dyebath contains 10% (owf) extract and 1% nonionic wetting agent (Lissapol N., owf) with liquor ratio of 50:1.

Optimum Time for Dyeing. In order to optimize time for dyeing, five dyebaths with optimized pH and temperature of dyeing with same liquor ratio of 50:1 were prepared. The temperature of these dyebaths was raised to optimized temperature and kept for fixation for 30, 45, 60, 90, and 120 minutes, respectively.

Optimum Concentration of Glauber’s Salt. To optimize the concentration of Glauber’s salt in acid dyeing, the dyebaths were prepared as mentioned above but only the concentration of Glauber’s salt was varied. The concentration of Glauber’s salt in the dyebaths was 0, 1, 4, 6, and 10% (owf), respectively, and the dyeing was carried out using optimized pH temperature and time conditions as optimized per earlier section.

2.2.3. Mordanting. Mordanting of lyocell with two metallic salts, namely, aluminum sulphate and ferrous sulphate, and two natural mordants, that is, *Harad* and *TKP*, was done by two mordanting techniques, namely, pre- and postmordanting. In case of pre- and postmordanting, the undyed or dyed samples were treated with different mordants (6% owf), at 60°C for 45 min.

2.2.4. After Treatment. All the samples were rinsed with water and then treated with 2 g/L nonionic detergent (Lissapol N) at 80°C for 15 min. Finally, the samples were washed thoroughly with water, squeezed, and dried.

2.3. Testing

2.3.1. Evaluation of Dyed Samples. The dyed samples were assessed for \( L^*a^*b^* \) colour coordinates and K/S values (illuminant D65/10° observer) on spectra scan 5100 (RT) spectrophotometer (Premier Colorsca n Instrument).

The light fastness of the dyed samples was tested on Fad-o-meter after partially exposing the samples to the xenon arc lamp for 16 hrs and graded for the colour change with the ratings. For wash fastness test, composite samples were prepared from dyed filaments, wool, and cotton yarns twisted together. Fastness test was carried out according to
ISO—2 test method. Samples were then separated, dried, and evaluated for the rating in terms of colour change.

2.3.2. Evaluation of Antimicrobial Activity by BPB Methods. For testing of white or light-coloured goods, 0.025% solution of bromophenol Blue (BPB) in distilled water was prepared. Few drops of saturated Na$_2$CO$_3$ solution were added per 100 mL of BPB solutions. The treated and untreated samples were soaked in 10 mL of above solution. Finally, we rinsed the samples in distilled water and observed the blue stain against bromophenol Blue colour test scale.

2.3.3. Evaluation of Antimicrobial Activity by AATCC 100 Methods. Antibacterial activity of the samples was evaluated by using AATCC 100 method using two bacteria, namely, *Staphylococcus aureus* (+Ve) (ATCC 6538) and *Escherichia coli* (−Ve) (ATCC 8739) as test bacteria. The antibacterial efficacy of the samples was also evaluated after 10 home launderings. The inoculums size was set at $1.5 \times 10^5$ cfu/mL and 1 gm of yarn in bundle form for each bacterium was used. Plate count spread plate technique was utilized for this bacterium efficiency test. Incubation period was 37°C for 24 hrs.

3. Results and Discussion

3.1. Effect of Various Parameters on Dyeing of Lyocell. To optimize dyeing of Lyocell with water and methanolic extracts of *Azadirachta indica* leaves various trials were conducted by varying dyebath pH, temperature, time of dyeing, and concentrations of chemicals, that is, concentration of salt. Results in terms of highest colour strength values are discussed in the following section.

3.1.1. Effect of Dyebath pH. Figure 1 shows the effect of pH on the colour strength obtained by *Azadirachta indica* leaves's aqueous as well as methanol extract. As can be observed, the best colour strength is obtained at pH 8.5 to slightly alkaline in lyocell. The effect of dye bath's pH can be attributed to the correlation between dye structure and lyocell.

3.1.2. Effect of Dyeing Temperature. The colour strength increases with increase of dyeing temperature and reaches a maximum value at boiling temperature as seen in Figure 2. This increase in dye uptake can be attributed to better dye exhaustion at higher temperatures.

3.1.3. Effect of Dyeing Time. Effect of dyeing time on colour strength is shown in Figure 3. The longer the dyeing time, the higher the colour strength until dye exhaustion attains equilibrium and there is decrease in the colour strength after further increase in time over 100 min. The decline in colour strength is attributed to the shift in equilibrium of coloring component from fabric into dye bath during longer dyeing time. Suitable dyeing time is 60 minutes.

3.2. Effect of Concentration of Dye on Colour Strength Value and Fastness Properties. Table 1 shows the colour strength values and fastness ratings of *Azadirachta indica* extract (water and methanolic) applied on lyocell hanks. From the table, it can be seen that as the concentration of dye in dyebath increases, the colour strength value was also increased, irrespective of the dye used. Higher colour strength values were obtained in case of lyocell dyed with methanolic extract compared to the yarn dyed with water extract.

The fastness ratings of dyed lyocell yarn are given in Table 1. It reveals that the washing fastness of dyed yarn was quite satisfactory to the decrease in concentration of dye in dyebath and increase in the washing fastness grade irrespective of *Neem* extract used. Generally, the wash fastness ratings, ranging from 3/4 to 4, were obtained in terms of colour change, which was categorized as good to very good washing fastness. The light fastness ratings obtained were in the range of 4 to 5/6 which was moderate to satisfactory.

![Figure 1: Effect of dyeing pH on relative colour strength.](image1)

![Figure 2: Effect of dyeing temperature on relative colour strength.](image2)
Table 1: Effect of concentration on relative colour strength value and fastness properties lyocell dyed with *Azadirachta indica* dye.

| Dye                                      | Concentration of dye % (owf) | Colour strength value (K/S) | L*   | a*   | b*   | Fastness ratings |
|------------------------------------------|------------------------------|-----------------------------|------|------|------|------------------|
| Water extract of *Azadirachta indica* leaves | 5                            | 1.02                        | 79.26| -1.12| 14.93| 4/5              |
|                                          | 10                           | 1.98                        | 73.43| 2.29 | 13.25| 3/4              |
|                                          | 15                           | 3.86                        | 68.32| 3.87 | 15.13| 3/4 5            |
| Methanolic extract of *Azadirachta indica* leaves | 5                            | 1.09                        | 71.62| -3.96| 34.73| 4/5 5            |
|                                          | 10                           | 2.23                        | 67.43| -9.82| 33.81| 3/4 4/5          |
|                                          | 15                           | 4.15                        | 53.26| -8.04| 32.34| 3/4 4/5          |

Table 2: Colour strength (K/S values) of lyocell dyed with water and methanolic extracts of *Azadirachta indica* leaves using different mordants by exhaust technique of dyeing.

| Process                  | Mordant (6% owf) | Colour strength (K/S values) |
|--------------------------|------------------|-----------------------------|
|                          | Water extract    | Methanolic extract          |
|                          | 5% 10% 15%       | 5% 10% 15%                  |
| Control                  | —                | 0.83 1.12 1.49              | 0.77 1.06 1.31 |
| Premordanting            | TKP              | 1.11 1.71 1.79              | 1.16 1.20 1.43 |
|                          | Harad            | 1.01 1.13 1.21              | 1.22 1.36 1.37 |
|                          | FeSO₄            | 2.36 3.34 4.09              | 1.55 2.74 3.57 |
|                          | Al₂(SO₄)₃        | 0.64 0.88 1.78              | 0.95 1.94 3.66 |
| Postmordanting           | TKP              | 1.71 1.91 2.47              | 1.61 3.56 4.38 |
|                          | Harad            | 1.37 1.56 1.67              | 1.97 2.08 2.27 |
|                          | FeSO₄            | 2.11 3.76 3.87              | 2.01 2.39 4.70 |
|                          | Al₂(SO₄)₃        | 0.87 1.76 3.12              | 1.14 1.90 2.21 |

Note: Concentration of dye 5, 10, and 15% owf.

3.3. Effect of Mordant and Mordanting Techniques on Colour Strength Value. From Table 2, it can be seen that the colour strength value increases as the concentration of dye increases in the dyebath. In case of samples where metallic salts were used, the colour strength (in terms of K/S value) is increased compared to the samples dyed without metallic salt except in case of Al₂(SO₄)₃ as metallic salt where the colour strength is decreased. The maximum colour strength value occurs with the sample where ferrous sulphate was used as metallic salt.

From the result it can also be seen that the postmordanting process gives maximum K/S values compared to the premordanting processes.

3.4. Effect of Mordant on Colour Hue. In all the cases the colour obtained on lyocell is ranging from pale green to medium green using methanol extract and light brown by water extract of *Azadirachta indica* leaves as seen from the
Table 3: Color coordinates of lyocell yarn dyed with water and methanol extracts of *Azadirachta indica* leaves.

| Dye Process | Mordant | \( L^* \) | \( a^* \) | \( b^* \) |
|-------------|---------|----------|----------|----------|
| Control     | —       | 57.43    | -11.69   | 32.52    |
| Premordanting | TKP     | 75.315   | -0.556   | 19.145   |
|              | Harad   | 68.715   | -1.761   | 22.951   |
| Methanol extract | FeSO_4   | 68.005   | 1.295    | 17.089   |
|              | Al_2(SO_4)_3 | 78.858 | -2.803   | 20.986   |
| Postmordanting | TKP     | 69.385   | -2.994   | 20.235   |
|              | Harad   | 75.333   | 0.688    | 20.088   |
| Water extract | FeSO_4   | 63.070   | 0.422    | 11.019   |
|              | Al_2(SO_4)_3 | 78.068 | -4.826   | 17.641   |

Note: Concentration of dye 10% owf.

It was also observed from the table that the use of metallic salt except Al_2(SO_4)_3 increases the colour strength value but cannot alter the tone or hue of the colorant extracted from *Azadirachta indica* leaves.

3.5. Effect of Mordant on Fastness Properties. Light and washing fastness ratings of lyocell yarn dyed with water and methanol extract of *Azadirachta indica* leaves using exhaust dyeing method are shown in Table 4.

Table 4 also shows the fastness ratings of dyed samples. The samples treated without metallic salt show poor to satisfactory light fastness and good to very good washing fastness properties. From Table 4, it can be seen that the metallic salts may alter the fastness ratings by 1 or 2 points.

3.6. Probable Mechanism of Dyeing. From the above results, it can be seen that a satisfactory depth is attend with both water and methanolic extracts of *Azadirachta indica* leaves used in the study. The dyestuff bonding on lyocell fibre can be explained on the basis of structural formula of the fibre.

3.6.1. FTIR Spectral Analysis. The lyocell fibre, undyed (UNT) and dyed with methanolic extract of *Azadirachta indica* leaves, was analyzed by the Fourier transform infrared spectra and illustrated in Figure 5.

The band near 1156 cm\(^{-1}\), representing the antisymmetric bridge stretching of the C-O-C groups, the band near 1312 cm\(^{-1}\), representing –CH\(_2\)– wagging vibrations, and the 894 cm\(^{-1}\), being characteristic of \(\beta\) linkages, did not alter after dyeing with methanolic extract of *Azadirachta indica* leaves. The bands near 3351 cm\(^{-1}\) represent –OH vibrations. The broad peak that occurs at 3400–3200 cm\(^{-1}\) is associated with –OH stretching in hydroxyl groups originating mainly from cellulose. The peak at 2917 cm\(^{-1}\) is assigned to –CH stretching in –CH\(_2\)– groups. The peak at 1023 cm\(^{-1}\) is associated with both the C–O stretch in cellulose and the C–O deformation in the primary alcohols of lignin. Different characteristic peaks seen in the figures confirmed the structure of untreated lyocell yarn and the yarn dyed with methanolic extract of *Azadirachta indica* leaves dye as well.
Table 4: Fastness ratings of lyocell yarn dyed with water and methanol extracts of Azadirachta indica leaves.

| Dye Process       | Mordant   | LF | Fastness test |
|-------------------|-----------|----|---------------|
| Control           | —         | 5  | 3             |
| Premordanting     | TKP       | 5  | 4             |
|                   | Harad     | 5  | 4             |
| Methanol extract  | FeSO₄     | 4  | 4             |
|                   | Al₂(SO₄)₃| 5  | 3-4           |
|                   | TKP       | 5  | 3-4           |
|                   | Harad     | 5  | 3             |
|                   | FeSO₄     | 4  | 3-4           |
|                   | Al₂(SO₄)₃| 5  | 3             |
| Post-mordanting   | TKP       | 5  | 4-5           |
|                   | Harad     | 5  | 4             |
| Water extract     | FeSO₄     | 5  | 4             |
|                   | Al₂(SO₄)₃| 5  | 4-5           |
|                   | TKP       | 5  | 4             |
|                   | Harad     | 5  | 4-5           |
|                   | FeSO₄     | 4-5| 4             |
|                   | Al₂(SO₄)₃| 5  | 4-5           |

Note: Concentration of dye 10% owf, LF = light fastness, WF = wash fastness.

3.6.2. Attachment of Azadirachta indica Leaves Extract to Lyocell. The FTIR spectrum of methanolic extract of Azadirachta indica leaves. Major bands in 1,800–1,600 cm⁻¹ region were observed. The band at ~1,716 cm⁻¹ was assigned to stretching vibrations of the two ester-carbonyl groups in the chlorophyll molecule. The band at ~1,635 cm⁻¹ was assigned to the stretching vibration of aggregated keto groups in the chlorophyll molecule. The band close to 1,600 cm⁻¹ was assigned to skeletal C=C and C=N stretching vibrations of the aromatic system in the chlorophyll molecule. The peaks at these characteristics frequencies or nearby one observed in the FTIR spectra of the Neem treated fabric can be used as an indication of terpenoids structures, that is, nimbin, nalmannin, and so forth, being chemically attached on to the fabric. Stretching bands at 668 cm⁻¹, 1156 cm⁻¹, 1261 cm⁻¹, 2360 cm⁻¹ and 3989 cm⁻¹ in Azadirachta indica extract treated fabric clearly indicate that the active ingredients of the Azadirachta indica extract have been attached on to the lyocell.

3.7. Antimicrobial Efficiency of Dyed Samples. The comparative results of antimicrobial property of samples with and without treatment with extract are shown in Table 5. The ratings are given by symbols, “−” indicates poor antimicrobial efficiency and “+” indicates good antimicrobial efficiency and so on. Table 5, shows the efficiency of treatment on lyocell yarn. From the results, it can be seen that the samples treated with 15% (owf) concentration of Azadirachta indica leaves extract exhibited good antimicrobial property as per BPB scale. Efficiency of the treatment can be further enhanced by the addition of copper salt as mordant in the dyebath.

Figure 6 shows the antibacterial activity of untreated and yarns treated with methanolic extract (15% owf) of Azadirachta indica leaves evaluated by using AATCC 100 method. The treated samples before washing showed excellent antibacterial activity against both Staphylococcus aureus (100%) and Escherichia coli (99.94%) as compared to untreated sample. An excellent level of antibacterial activity was observed against each of the test bacterial species. In each case a greater than four to five log reduction in bacterial numbers on treated compared to untreated samples was observed. The antibacterial efficacy of sample only dyed with methanolic extract exhibits 99.82% efficacy against Gram-negative bacterium E. coli and 99.36% efficacy against S. aureus (which is a Gram-positive bacterium) even after 10 washes. This clearly indicates that the methanolic extract acts as bactericidal.

4. Conclusion

Water and methanolic extracts of Azadirachta indica leaves can successfully be utilized for the colouration of lyocell yarn with moderate fastness properties. The treatment with water and methanolic extracts not only enhanced the aesthetic value but also impart bioactivity to lyocell yarn. The FTIR analysis results indicate various groups in the extract, namely, chlorophyll which is responsible for green colour and attachment of antimicrobial compounds, that is, nimbin, nimbinene, nimbandiol, immobile, nimocinol, quercetin,
Table 5: Efficiency of antimicrobial treatment according to bromophenol Blue test scale.

| Sample                          | Concentration of extract % (owf) | Mordent       | Symbolic rating BPB scale | Remark                  |
|---------------------------------|----------------------------------|---------------|---------------------------|-------------------------|
| Undyed lyocell                  | —                                | —             | —                         | Untreated               |
| 5                               | —                                | —             | —                         | Undertreated            |
| 10                              | —                                | —             | — +                       | Slightly undertreated   |
| 15                              | CuSO⁴                           | +++           |                           | Good treatment          |
| 15                              | Al₂(SO₄)_₃                      | ++            |                           | Slightly overtreated    |
| 15                              | TKP                              | +             |                           | Good treatment          |
| 15                              | Harad                            | +             |                           | Good treatment          |
| Lyocell yarn dyed with water extract | 5                                | —             | —                         | Undertreated            |
| 10                              | —                                | —             | — +                       | Slightly undertreated   |
| 15                              | CuSO⁴                           | +++           |                           | Good treatment          |
| 15                              | Al₂(SO₄)_₃                      | ++            |                           | Good treatment          |
| 15                              | TKP                              | +             |                           | Good treatment          |
| 15                              | Harad                            | +             |                           | Good treatment          |

Note: Concentration of mordant 6% owf using premordanting technique.

Untreated sample plate
("0hr" contact time) | Treated sample plate
("24hrs" contact time) | After 10 washes
("24 hrs" contact time)

**Escherichia coli** (−Ve) (ATCC 8739)

| Untreated sample plate | Treated sample plate | After 10 washes |
|------------------------|----------------------|-----------------|
| 1.65 × 10^5 cfu/mL     | 1 × 10^2 cfu/mL      | 3 × 10^2 cfu/mL |

**Staphylococcus aureus** (Ve) (ATCC 6538)

| Untreated sample plate | Treated sample plate | After 10 washes |
|------------------------|----------------------|-----------------|
| 1.4 × 10^5 cfu/mL      | 0 cfu/mL             | >9 × 10^2 cfu/mL |

Figure 6: Antimicrobial activity of untreated, treated, and after 10 washes samples against *E. coli* and *S. aureus* using AATCC 100 method.
beta-sitosterol, and so forth, being chemically attached on to the yarn. 15% owm methanolic extract concentration in finishing bath was sufficient to impart high antibacterial activity (99%) to the lyocell yarn.

Finally, the lyocell yarns treated with extract of Azadirachta indica leaves can not only be utilized in fashion industry to enhance the aesthetic value but can also utilize medicinal textiles.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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