Antimicrobial activity of cotton fabric treated with *Quercus infectoria* extract

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Cotton fabric has been treated with the tannin-rich extract of *Quercus infectoria* (QI) plant in combination with alum, copper and ferrous mordants and then tested for antimicrobial activity against Gram-positive and Gram-negative bacteria. QI extract shows good activity at 12% concentration (owf), inhibiting the microbial growth by 45-60%. The microbial growth inhibition increases to 70-90% when alum and copper sulphate are used for mordanting. However, the antimicrobial activity is completely lost when ferrous sulphate is used. The activity of samples treated with QI alone is lost completely after 5 launderings while the mordanted samples retain nearly 100% activity up to 5 launderings, indicating that the mordants help to make the effect durable. The study also shows that the cotton textiles can be successfully treated with QI to produce bioactive textiles from natural ecofriendly materials. QI is a viable alternative to synthetic antimicrobial agents for use in hospital textiles and an effective antiodour agent for use in sports and household textiles.

**Keywords**: Antimicrobial activity, Bioactive textiles, Cotton, Natural Dyes, *Quercus infectoria*

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1 Introduction

Traditional medicinal systems, like the *Ayurveda* and *Siddha*, mention the use of plants in treatment of various human ailments. India has about 45,000 plant species and among them, several thousand have been claimed to possess medicinal properties. Over the past decade, substantial progress has been made in research on the natural products for the treatment of several dreaded diseases, like AIDS and cancer.\(^1\)\(^2\) Although a significant number of studies have used known purified plant chemicals, very few screening programmes have been initiated on crude plant materials. Even so, we have barely scraped the surface in our efforts to exploit the plant world for antimicrobials (antiviral, antibacterial and antifungal compounds). Recognising the importance of plant materials as antimicrobial agents, research has been initiated in the area of producing bioactive textiles for the protection of wearer from common microbes causing cross infections. Natural antimicrobial agents are non-toxic and non-allergenic and do not cause the problems of microbial resistance.

*Quercus infectoria* (QI) has been used to treat cotton fabrics since time immemorial. As natural dyes show no direct affinity for cotton fabric, all such textiles are first treated with myrobalan (*harda*) before dyeing with any vegetable dye. This product serves more as a mordant that helps to fix the dye on cotton by providing hydroxyl groups, which act as dye sites. The galls of QI contain tannic acid (gallo tannic acid) as the principal constituent (50-70%). The tannic acid and gallic acid extracted from the galls are powerful astringents.\(^3\) Redwane and Lazrek\(^4\) reported the efficacy of extracts and fractions of QI galls as larvicidal agents and their possible use in biological control of the urban nuisance mosquito. The antimicrobial properties of tannins have also been reported.\(^5\)

Though ethnobotanical studies have established the efficacy of QI extract as an effective antimicrobial agent, no studies have been reported on the treatment of textiles with these materials. QI has good affinity for cotton and can be easily exhausted. The present work is, therefore, aimed at studying the antimicrobial properties of cotton fabric treated with QI against selected bacterial species known to cause cross infections in hospitals and also responsible for causing unpleasant odours in textiles.

2 Materials and Methods

Plain weave cotton fabric, having the specifications 107 g/m\(^2\) weight, 40 ends/cm and 35 picks/cm, was used for the study. Commercial sample of *Quercus infectoria* (QI) was obtained from Alps Industries Ltd, Sahibabad, India. Antimicrobial agent Fabshield...
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AEM 5700 by Aegis Chemicals was obtained from Rossari Industries Ltd, India and used as a reference. All other chemicals used were of LR grade.

Gram-negative bacteria Escherichia coli (E.coli) and Gram-positive bacteria Bacillus subtilis (B.subtilis), procured from ATCC (American Type Culture Collection) USA, were used for the study. Agar powder and peptone, both of AR grade, were used.

Fabric, glassware and culture media were autoclaved to sterilize them. Static and rotary shaker type incubator, manufactured by Orbital Ltd, was used. Electronic colony counter was used to count the microbial colonies. A sterile chamber equipped with laminar airflow, UV lamp and a burner was used for inoculation. Julabo SW22 bath of horizontal shaker type with automatic temperature control was used for treating cotton fabric with QI and for mordanting. Launderometer was used for washing the samples.

Optical density was recorded on the Biochrom UV-Vis spectrophotometer. SEM analysis was carried out on Cambridge Sterioscan 360 at a scale of 5-10 mm.

2.1 Determination of Antimicrobial Activity of Dyed Fabrics

Treatment with QI was carried out using material-to-liquor ratio of 1:30 at 60°C and neutral pH. To study the effect of mordants, alum [K2Al2(SO4)4], CuSO4 and FeSO4 were used at 1% and 5% (owf) concentrations. Fabric was pretreated with alum at a temperature of 80°C, and then treated with QI for 45 min. Samples were post mordanted with copper sulphate after treatment with QI. Treated fabric samples were rinsed with cold water, soaped with 0.5 g/L non-ionic detergent (Lissapol N) and dried.

The antibacterial activity was assessed by qualitative testing of fabric using the parallel streak method (AATCC Test Method 147-1993). Untreated cotton and cotton treated with 12% QI in combination with alum, copper sulphate and ferrous sulphate were placed on an agar plate streaked with two parallel streaks of microbial inoculum. The plates were observed after 24 h of incubation. Samples which have antimicrobial activity do not show any growth under and around the samples while streaks of microbial growth can be observed under non-active fabrics.

For quantifying the antimicrobial activity the agar plate method (AATCC 100-1993) was used. Fabric samples of defined weight (0.06g) and dimension (1 inch^2) were sterilized and kept in liquid culture media. Bacterial culture (100μl) was added to the liquid media and then it was incubated at 37°C for 24 h. Microbial inhibition was determined by the reduction in number of colony forming units (CFU) with respect to untreated control sample using the following formula:

\[ \text{Reduction in microbial colonies} = \left( \frac{B - A}{B} \right) \times 100 \]  

where \( A \) is the CFU/ml for the treated sample after 24h incubation time; and \( B \), the CFU/ml for the untreated sample incubated under identical conditions.

Scoured cotton was also treated with 0.5% (owf) Fabshield (commercial antimicrobial agent) at 50°C and 4.5 pH for 20 min, followed by curing at 130-135°C. Dyed samples were tested for the durability of treatment using ISO II test for wash fastness. Washed samples were tested for the retention of antimicrobial properties after single and 5 launderings.

3 Results and Discussion

For qualitative assessment of biological activity, cotton samples treated with QI extract with and without mordants were subjected to the parallel streak test. Results of the test are shown in Table 1. Untreated cotton samples show clear growth of microbes under them with no zone of inhibition, indicating that the cotton fabric by itself does not inhibit microbial activity. Treatments with QI alone and in combination with alum and copper mordants inhibit microbial growth as is evident from the absence of growth under all treated samples.

<table>
<thead>
<tr>
<th>Cotton fabric</th>
<th>Growth</th>
<th>Zone of inhibition, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.coli</td>
<td>B.subtilis</td>
</tr>
<tr>
<td>Untreated cotton</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>Fabshield treated</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>Treated with QI</td>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>QI + 1% Alum</td>
<td>Nil</td>
<td>1</td>
</tr>
<tr>
<td>QI + 5% Alum</td>
<td>Nil</td>
<td>0.5</td>
</tr>
<tr>
<td>QI + 1% CuSO4</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>QI + 5% CuSO4</td>
<td>Nil</td>
<td>1</td>
</tr>
<tr>
<td>QI + 1% FeSO4</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>QI + 5% FeSO4</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

+ sign shows positive growth under fabric.

Nil — No growth under fabric.
However, the samples treated with ferrous salt show microbial growth. Maximum zone of inhibition is observed for the sample treated with QI alone (Fig. 1). Sample treated with commercial product Fabshield shows no growth and no zone.

Lack of a zone of inhibition in this test does not necessarily mean an absence of activity. A zone is generally shown by antimicrobial agents that are ‘leaching type’, i.e. they leach out of the fabric and kill the microbes present on as well as around the treated fabric. Generally, the bigger is the zone the higher is the activity. It also means that the treatment though effective is non-durable and that the activity would be reduced with each wash. Such antimicrobial treatments are, therefore, not preferred. It is observed in this study that when cotton is treated with QI alone it leaches out and gives a zone. When a mordant is used, the dye is complexed with the mordant by formation of coordinate bonds and is thus rendered insoluble and hence it cannot leach out to give a zone. The dye now acts as an effective antimicrobial agent of the ‘bound type’. This effect is seen in case of samples treated with Fabshield and QI complexed with alum and copper sulphate. The use of ferrous salt suppresses the activity of QI, most likely due to the iron binding capacity of tannins. This leads to the capping of free phenolic –OH groups on QI which are primarily responsible for antimicrobial activity. In the next set of experiments, quantitative assessment of the treated samples was carried out by the agar plate test.

3.1 Quantitative Assessment of Biological Activity of Dyed Fabric

Quantitative estimation of biological activity was carried out to determine the minimum inhibitory concentration (MIC) of dye on fabric. Estimation was done by counting the colony forming units in the culture incubated with treated samples for 24 h. The results are shown in Fig. 2. It can be observed from the figure that while cotton treated with 6% QI shows a very low extent of inhibition (12%), the significant enhancement in activity (45% reduction) occurs against *E.coli* when 12% QI is used. On addition of 5% alum, the inhibition improved to 73% and with 5% copper to 57%. With ferrous salt, however, there is no activity at all. Fabshield treated cotton shows 75% inhibition of microbial colonies. This confirms the findings of the qualitative test conducted earlier. Hence, it can be inferred that 12% QI in combination with 5% alum can act as a good antimicrobial agent against Gram-negative bacteria *E.coli*.

![Fig. 1 — Parallel streak test on cotton against *S.aureus*](image)  
(a)untreated sample and (b) sample treated with 12%QI

**Fig. 1** — Parallel streak test on cotton against *S.aureus*  
(a)untreated sample and (b) sample treated with 12%QI

![Fig. 2 — Antimicrobial activity of cotton fabric treated with QI against *E.coli*](image)

**Fig. 2** — Antimicrobial activity of cotton fabric treated with QI against *E.coli*

![Fig. 3 — Antimicrobial activity of cotton fabric treated with QI on *B.subtilis*](image)

**Fig. 3** — Antimicrobial activity of cotton fabric treated with QI on *B.subtilis*

QI shows similar results against the Gram-positive bacteria *B.subtilis*. In general, the activity is higher as compared to that against *E.coli*. Ikigai *et al.* have also reported that the Gram-positive bacteria are more sensitive to the bactericidal effect of tannins than Gram-negative bacteria. Six per cent QI is found to be ineffective in controlling microbial growth but at 12% QI concentration it shows 60% inhibition against *B. subtilis* (Fig. 3). This improves to 84% and 89% in combination with 5% of alum and copper salts.
respectively. These findings are in agreement with the results of the qualitative test.

As reported earlier, the samples post mordanted with ferrous sulphate show no activity. This phenomenon can be explained as follows. QI is a tannin-rich dye and the biological activity of tannins is probably affected to a great extent by the molar content and spatial configuration of the \(-\)phenolic hydroxyl groups.\(^8\) The exact mechanism of antimicrobial activity is not known but many studies report that it is most likely due to the disruption of membrane integrity.\(^9\) The iron binding activity of tannins has been well studied.\(^{10,11}\) Since the \(-\)phenolic groups of tannin are involved in complex formation with iron salts, the antimicrobial activity is completely lost when iron is used as a mordant with QI. SEM photographs of \(B.\) subtilis growth and its inhibition by QI are shown in Fig.4.

### 3.2 Durability of Antimicrobial Effect

A key issue to be considered in any finishing treatment is the durability to washing of the functional treatment. Temporary finishes are good only for disposable articles. For all other applications, the finish should be reasonably resistant to washing. To test the durability to laundering, biological activity of dyed cotton was tested after 1 and 5 launderings. As the effect in this case is due to a polyphenolic compound having good affinity for cellulose, it is expected to have high durability. The findings are reported in Table 2.

It can be observed that the samples treated with QI alone retain about 50% activity after the first laundering. But the activity is almost completely lost after 5 launderings. However, the samples treated with QI in combination with 5% of alum or 1% copper sulphate retain more than 75% activity even after 5 launderings. Hence, QI alone can be used to impart a temporary finish to cotton. This may be required for articles which do not need laundering as in disposables and hygiene market. However, if a finish durable to laundering is required, QI has to be used in combination with alum or copper mordants. Out of the two, alum has higher activity and poses no environmental hazard; it should be the preferred salt.

### 4 Conclusions

It is possible to produce cotton textiles with durable antimicrobial effect using the extract of the plant \(Quercus\) infectoria. Its activity has been tested against one Gram-negative and one Gram-positive bacterium, namely \(B.\) subtilis and \(E.\) coli respectively. 12% of QI extract (owf) is found to be the effective concentration against selected microbes. The activity is greatly enhanced when 5% alum or 1% copper

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**Table 2— Retention of antimicrobial activity by treated cotton after multiple launderings**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Cotton fabric</th>
<th>After single laundering</th>
<th>After 5 launderings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reduction in cfu %</td>
<td>Activity retention %</td>
</tr>
<tr>
<td>(B.) subtilis</td>
<td>Control</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Treated with</td>
<td>QI</td>
<td>33</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>QI +5% alum</td>
<td>83</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>QI + 1% CuSO(_4)</td>
<td>84.1</td>
<td>100</td>
</tr>
<tr>
<td>(E.) coli</td>
<td>Control</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Treated with</td>
<td>QI</td>
<td>55.6</td>
<td>65.4</td>
</tr>
<tr>
<td></td>
<td>QI + 5% alum</td>
<td>30.2</td>
<td>84.4</td>
</tr>
<tr>
<td></td>
<td>QI + 1% CuSO(_4)</td>
<td>5.6</td>
<td>75.1</td>
</tr>
</tbody>
</table>

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Fig. 4 — SEM photographs showing the growth of \(B.\) subtilis (a) untreated cotton and (b) cotton treated with 12% QI.
sulphate is used as a mordant. Studies on durability of the treatment show that the QI treatment is not durable to washing and the antimicrobial activity is lost completely after 5 launderings. However, samples mordanted as above show 80-100% retention of activity even after 5 washes. Hence, it may be said that the QI can be used successfully for treating cotton textiles as an alternative to the very expensive, synthetic and sometimes toxic antimicrobial agents available in the market today.

References