

# A Study on Antimicrobial Properties of Cotton Fabric Treated with Neem Leaf Extract

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**Abstract:** *This study focuses on the development of bacterial resistant cotton fabric using neem leaf extract. In this study, neem leaf extract is prepared in ethanol using Soxhlet extraction method. The identification of major compounds is done by using phytochemical test. The finish is applied in three concentrations (3g/l, 5g/l, 7g/l) on cotton fabric. Neem leaf antimicrobial finish has been imparted to the cotton fabric by pad-dry-cure method. The assessments tests for determining the antimicrobial performance for untreated and treated sample fabrics are carried out in accordance with ISO 20645. Qualitative analysis is carried out to measure the antimicrobial activity against gram positive bacteria i.e. S. aureus, B. subtilis, B. pumilus as well as gram negative bacteria i.e. E. coli, P. aeruginosa and C. albican. And then physical properties of untreated and treated cotton fabrics such as breaking strength and fabric stiffness are analysed. From the overall results it is seen that the neem treated sample with citric acid has a better antimicrobial activity than that with acetic acid.*

**Keywords:** *neem leaf extract, antimicrobial agent, Soxhlet, Gram positive, Gram negative.*

## 1. INTRODUCTION:

Textile materials are good carries of various types of microorganisms and can cause health related problems to the wearer. These microorganisms create problems in textile, including discolouration, stains and fibre damage, unpleasant odour and a slick, slimy feel [1]. In order to impart the required functional properties to the fibre or fabric, it is customary to subject the material to different type of physical and chemical treatments [2]. In the abundance of various finishes, antimicrobial finish is becoming one of the important finishes since people take much care about health and hygiene. Hygiene has acquired more importance in recent years [3].

Some of the herbal compounds obtained from plants are well known from time immemorial as antibacterial and antifungal products. These plants and tree products are applied directly on skin or wounds as paste or incantion either for skin care or wound healing. These natural products are abundantly available in nature and are widely distributed. They are cheap and not processed and can be used as raw materials for required applications. The stem, bark, leaf, root and tuber of the plants and trees can be used for special application [4].

In this study, an eco-friendly and effective antibacterial agent is extracted from neem leaves and applied on cotton fabric to obtain the antimicrobial treated fabric.

## 2. EXPERIMENTAL PROCEDURE:

### 2.1 Collection of Samples

In this study, neem leaves are collected from the tropical region of Minbu Township, Magway Division in Myanmar, during the period from June to December and the bleached cotton plain woven fabric is collected from the local market.

### 2.2 Preparation of Antimicrobial Agent from Neem Leaves

After collection of neem leaves sample, they are washed thoroughly two to three times with water. The leaves are dried at room temperature for seven days. After complete drying, the leaves are crushed into coarse powder by using a wooden grinder.

### 2.3 Extraction of Antimicrobial Agent from Neem Leaves

In this study, the active compounds from the dried leaves powder of neem are extracted by Soxhlet extraction method. After that, the solution is cooled and filtrated through the filter paper. The liquid extract containing active constituents is concentrated to one fifth volume at 50°C under reduced pressure by means of rotatory evaporator. The concentrated solution is then defatted with petroleum ether by shaking to remove fatty oil and lipid compounds. After that, the extracted solution containing active compounds is obtained. And then it is evaporated at 50°C by using water-bath to obtain a dried neem leaf extract.

## 2.4 Preliminary Phytochemical Tests on the Neem Leaf Extract

The qualitative phytochemical tests are performed to investigate the types of chemical compounds in the neem leaf extract. These tests are performed according to the procedure prescribed in the Text book of Pharmacognogy, Pharmacopoeia of India, Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis.

## 2.5 Antibacterial Finish on Cotton Fabric

In this study, antimicrobial finish on cotton fabric is carried out by using pad-dry-cure method. Firstly, the cotton fabrics are immersed into the neem leaf extracted solution at various concentrations of 3 g/l, 5 g/l, and 7 g/l, respectively for thirty minutes. Acetic acid is added to maintain the level of pH 5.5. Material to liquor ratio of 1:40 is used based on the weight of fabric. After that, this sample is taken out and padded on auto padder mangle. The fabric is then dried at 80°C for five minutes and cured at 120°C for three minutes on chamber dryer.

In order to achieve the better antimicrobial activity on the treated cotton fabric, 10% citric acid and 10% acetic acid based on the weight of the sample are used as crosslinking agent, respectively. The treated fabrics are immersed into the acid solution. This solution is heated to a temperature of 50°C for five minutes and then the fabrics are padded, dried at 80°C for five minutes and cured at 110°C, 110°C and 120°C for three minutes in treatment I, II, and III respectively. The classification of antimicrobial treated sample fabrics is shown in Table 2.1.

Table 2.1. Classification of Antimicrobial Treated Samples

Sample Code	Concentration (g/l)	Crosslinking Agent (acid)	Curing Temperature (°C)	Treatment
A <sub>1</sub>	3	Citric	110	I
B <sub>1</sub>	5	Citric	110	I
C <sub>1</sub>	7	Citric	110	I
A <sub>2</sub>	3	Acetic	110	II
B <sub>2</sub>	5	Acetic	110	II
C <sub>2</sub>	7	Acetic	110	II
A <sub>11</sub>	3	Citric	120	III
B <sub>11</sub>	5	Citric	120	III
C <sub>11</sub>	7	Citric	120	III

## 2.6 Preparation of Treated Samples and Discs for Antimicrobial Activity Test

The antimicrobial activity of treated cotton fabric is determined by agar disc diffusion method described by Cruickshank 1975. Nutrient agar (4.2 g) and agar (1 g) are dissolved in 150 ml distilled water. The nutrient agar mediums are separated into six flask tubes equally. All flasks are autoclaved at 121°C for 20 minutes and cooled in water bath at 60°C. After cooling, bacteria suspension of each bacterial and fungal strain (0.02 ml) is added and poured into sterilized petridishes. The seeded plates were allowed to dry at room temperature for 20 minutes. After that, the sample of 15 mm in diameter is placed on each of the plate and incubated at 37°C for 24 hours. After overnight incubation, antibacterial activity of each treated sample is determined from the zone of inhibition diameter.

## 3. RESULTS AND DISCUSSIONS:

### 3.1 Phytochemical Investigation on Neem Leaf Extract

Phytochemical tests are carried out to identify whether the presence or absence of antimicrobial compounds in the neem leaf extract. Phytochemical screening of neem leaf extract shows the presence of alkaloids,  $\alpha$ -amino acids, terpenoids, glycosides, reducing sugar, phenols, saponins, carbohydrate and flavonoids. Among these compounds, alkaloids, glycosides, terpenoids, flavonoids and saponins are antibiotic principle of neem trees which are actually the defensive mechanism of the plants against different pathogens.

### 3.2 Test Results of Antimicrobial activity on Untreated and Treated Fabrics

Screening of antimicrobial activity on untreated and treated sample fabrics have been done by agar disc diffusion method. The assessment tests for determining the antimicrobial performance for untreated and treated sample fabrics are carried out in accordance with ISO 20645.

The test results of antimicrobial activity on untreated and treated sample fabrics for treatment I, II and III are described in Table 3.1, Table 3.2 and Table 3.3.

Table 3.1. Test Results of Antimicrobial Activity on Untreated and Treated Samples for Treatment I

Sample Code	Antimicrobial Activity on Organisms					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. pumilus</i>	<i>C. albicans</i>	<i>E. coli</i>
A <sub>1</sub>	+	+	+	+	+	+
B <sub>1</sub>	++	++	++	++	++	++
C <sub>1</sub>	+++	+++	+++	+++	+++	+++
Untreated	-	-	-	-	-	-

Table 3.2. Test Results of Antimicrobial Activity on Untreated and Treated Samples for Treatment II

Sample Code	Antimicrobial Activity on Organisms					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. pumilus</i>	<i>C. albicans</i>	<i>E. coli</i>
A <sub>2</sub>	+	-	-	+	+	-
B <sub>2</sub>	+	+	+	+	+	+
C <sub>2</sub>	+	+	+	+	+	+
Untreated	-	-	-	-	-	-

Table 3.3. Test Results of Antimicrobial Activity on Untreated and Treated Sample Fabrics for Treatment III

Sample Code	Antimicrobial Activity on Organisms					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. pumilus</i>	<i>C. albicans</i>	<i>E. coli</i>
A <sub>11</sub>	+	+	+	+	+	+
B <sub>11</sub>	++	++	++	++	++	++
C <sub>11</sub>	+++	+++	+++	+++	+++	+++
Untreated	-	-	-	-	-	-

+++ - Maximum antibacterial activity  
 ++ - Moderate antibacterial activity  
 + - Minimum antibacterial activity  
 - - No activity

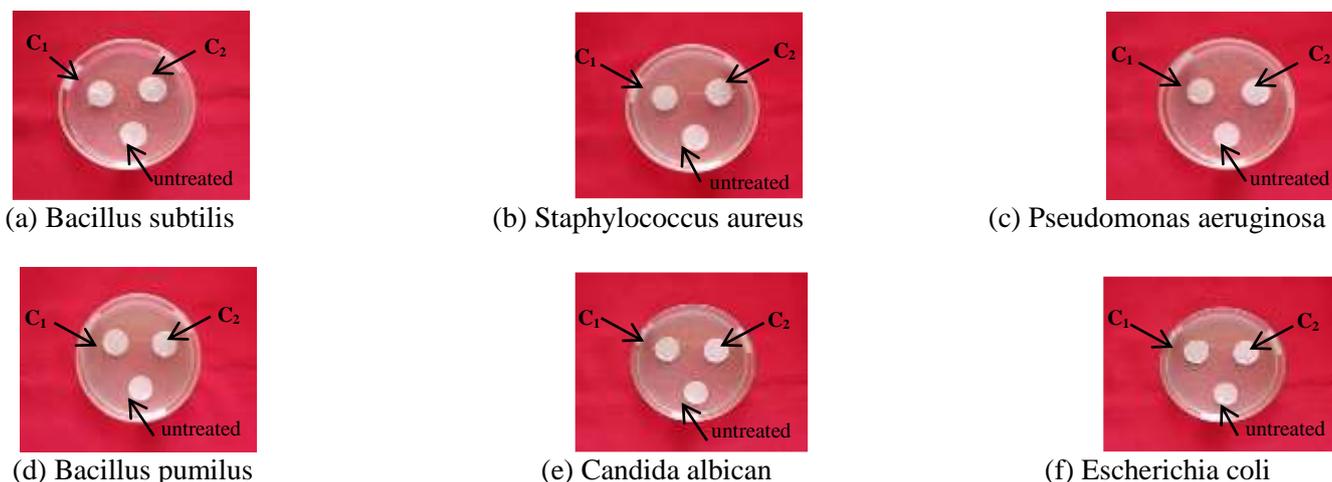


Figure 3.1. Antimicrobial Activity on Untreated and Treated Samples with 7 g/l Concentration of Extracted Solution for Treatment I and II

Figure 3.1 shows the antimicrobial activity of untreated and treated sample fabrics with 7 g/l concentrations of extracted solution on six organisms for treatment I and II. According to the test results shown in Table 3.1 and Table 3.2, it is observed that citric acid is more effective than acetic acid to obtain antimicrobial activity. The reason for this is that citric acid is not easily volatile at the curing temperature of 110°C and 120°C because its boiling point is 310°C. However, acetic acid is readily volatile at these temperatures because its boiling point is 118.1°C and thus it cannot serve as a crosslinking agent for a long time. Because of this reason, the antimicrobial fabrics are only treated with citric acid in treatment III.

According to the results described in Table 3.1 and Table 3.3, it is found that the greater the concentration of extracted solution, the more effective the antimicrobial activity on cotton fabric. It can also be found that the sample treated with 7 g/l concentration of extracted solution has the excellent antibacterial activity in both treatment I and treatment III. Thus, the curing temperature of 110°C in treatment I should be used in order to save the energy consumption.

### 3.3 Comparison of Functional Properties of Untreated and Treated Cotton Fabric

Antimicrobial finishing treatment may influence on breaking strength and stiffness of the fabric. And so different finished samples are analysed and required tests are carried out at standard atmospheric condition (20±2°C, 65±2% RH) in the Laboratory of Textile Testing and Quality Control of the Department of Textile Engineering, Yangan Technological University. The summary of results is shown in Table 3.4.

Table 3.4. Summary of Test Results on Physical Properties of Untreated and Treated Samples

Sr. No.	Sample Code	Breaking Strength (kg)		Bending Length (cm)	
		Warp	Filling	Warp	Filling
1	Untreated	40.00	30.40	2.64	1.94
2	A <sub>1</sub>	49.20	32.00	2.59	1.90
3	B <sub>1</sub>	49.40	32.60	2.55	1.90
4	C <sub>1</sub>	49.80	32.80	2.53	1.89
5	A <sub>2</sub>	40.40	30.60	2.62	1.93
6	B <sub>2</sub>	43.20	30.80	2.61	1.92
7	C <sub>2</sub>	43.80	31.00	2.60	1.91
8	A <sub>11</sub>	43.60	30.80	2.61	1.92
9	B <sub>11</sub>	45.40	31.00	2.60	1.91
10	C <sub>11</sub>	47.80	31.60	2.60	1.91

#### (1) Fabric Breaking Strength

The breaking strength of the untreated and treated sample fabrics is measured by using grab method. The breaking strength of a fabric depends on the finishing treatment it has received.

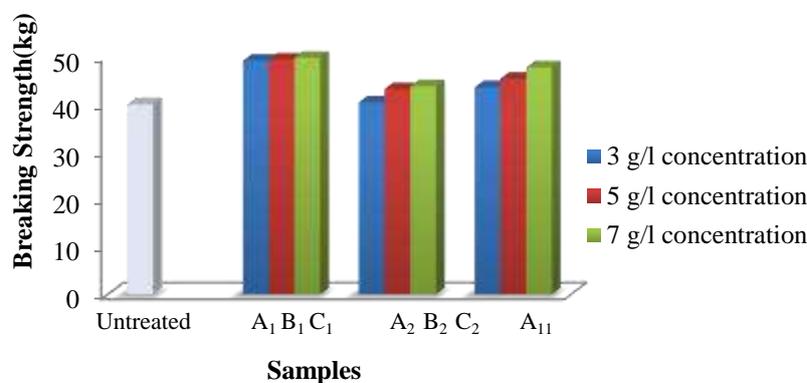


Figure 3.2. Effect of Treatment on the Breaking Strength of Untreated and Treated Samples in Warp Direction

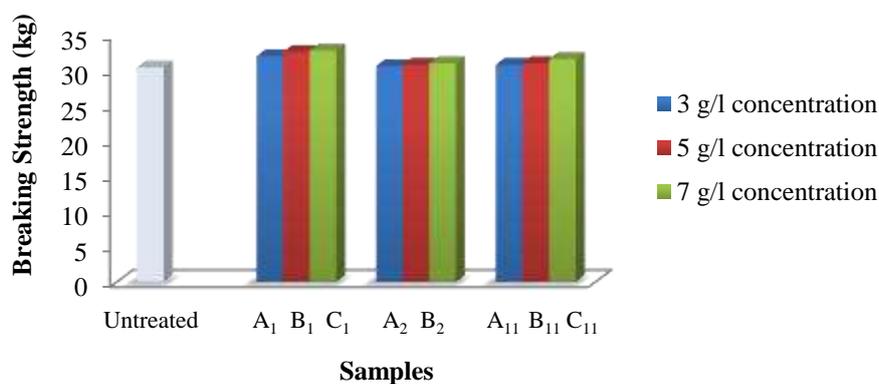


Figure 3.3. Effect of Treatment on the Breaking Strength of Untreated and Treated Samples in Filling Direction

Table 3.4 shows the results of the mean values of fabric breaking strength, in both warp and filling direction. All the treated sample fabrics show higher the breaking strength as compared to the untreated sample fabric in warp-way and weft-way due to the finishing treatment.

In Figure 3.2 and Figure 3.3, the effects of treatment are plotted against warp-way breaking strength and weft-way breaking strength, respectively. In treatment I, II and III, the breaking strengths of the treated sample fabrics slightly increase as the increase in the concentration of extracted solution. It is due to the fact that the neem leaf extract contains glucosides and amino acid. They compose of many hydrogen bonds that bind with the hydroxyl groups of the cellulose. The hydrogen bonding causes the molecules to draw closer to each other which increases the strength of the fibre and also aids in moisture absorption. It is learnt that the strength of the textile fibres is influenced by the moisture in the atmosphere when the strength test is made and natural vegetable fibres are stronger when they are wet.

(2) Fabric Stiffness

The stiffness of a fabric is defined as the resistance to bending. The stiffness is also dependent on the finishes which may be applied during the manufacturing process. The stiffness of the fabric is determined by using the Shirley Stiffness Tester. The stiffness of a fabric is also dependent to a high degree on the finishing treatment it has received.

Comparing the mean values of bending length as fabric stiffness for untreated and treated sample fabrics are described in Table 3.4, it is observed that the stiffness of the treated sample fabrics slightly decrease compared to the stiffness of the untreated sample fabric due to the finishing treatment.

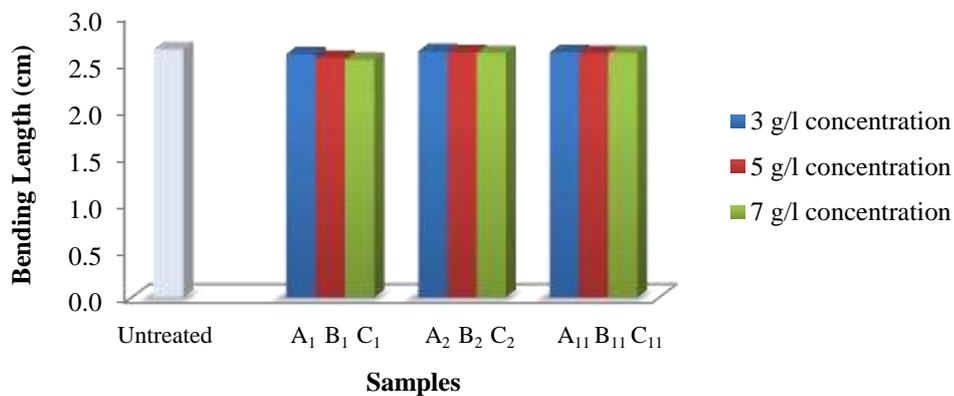


Figure 3.4. Effect of Treatment on the Fabric Stiffness of Untreated and Treated Samples in Warp Direction

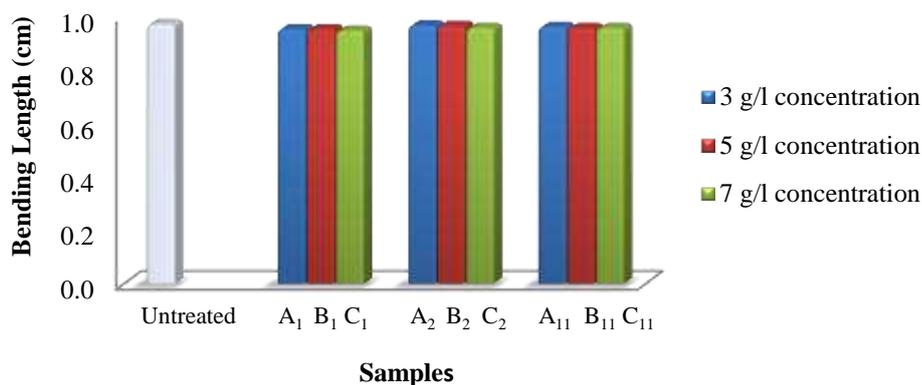


Figure 3.5. Effect of Treatment on the Fabric Stiffness of Untreated and Treated Samples in Filling Direction

Figure 3.4 and Figure 3.5 describe the effect of treatment on the fabric stiffness of untreated and treated sample fabrics. It is observed that the stiffness of the treated sample fabrics increases with the increase in the concentration of the neem leaf extracted solution. In warp direction, sample A<sub>1</sub> gives the highest fabric stiffness value while sample C<sub>1</sub> gives the lowest fabric stiffness value for treatment I. Also in filling direction, sample A<sub>1</sub> results in the highest fabric stiffness value while sample C<sub>1</sub> results in the lowest fabric stiffness value. Similarly, for treatment II and III, sample A<sub>2</sub> and A<sub>11</sub> have the highest values of fabric stiffness and sample C<sub>2</sub> and C<sub>11</sub> have the lowest values of fabric stiffness for

warp and filling direction respectively. The reason for this is that neem leaf extract composed of glucosides and  $\alpha$ -amino acids that contain hydrophilic groups; it can absorb the moisture very well and transmit this moisture to the surrounding atmosphere and causes the fabric more soften.

#### 4. CONCLUSIONS:

From this study, it is revealed that neem treated fabrics is eco-friendly, biodegradable and nontoxic to the skin. The phytochemical results show that neem leaf extract contains active constituents which exhibit antimicrobial activity against the six organisms *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Escherichia coli*, and *Candida albicans*.

According to the results, it is concluded that the neem treated sample with citric acid has a better antimicrobial activity than that with acetic acid. The higher the concentration of neem leaf extracted solution, the more effective the antimicrobial activity on cotton fabric. The antimicrobial finish with 7g/l concentration of extracted solution and citric acid gives the best antimicrobial activity on the treated cotton fabric among the treatments with the three concentrations of extracted solution (3g/l, 5g/l, and 7g/l). Since the results of antimicrobial activity on sample fabrics for treatment I and III are the same, the curing temperature 110°C should be used in order to save the energy consumption. Thus, the treatment I is the most preferable among the treatments for antimicrobial finish. According to the fabric analysis test on untreated and treated fabric samples, antimicrobial finish positively influences on the properties of the treated sample fabrics such as breaking strength and fabric stiffness.

#### 5. RECOMMENDATIONS:

Neem extract shows good antimicrobial activity on cotton fabric, and so it is recommended that the neem treated cotton fabric should be used for medical applications such as patient clothing, pillows cases, table cloths and bed sheets.

It is suggested that the durable antimicrobial fabrics should be proceeded on cotton fabric by washing test as for future work. And then, it is also recommended to investigate the antimicrobial activity of neem on cotton fabric for other test organism such as *Vibrio cholera*, *Shigella boydii*, and *Salmonella typhi*.

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