

Mechanically Robust and Antimicrobial Cotton Fibers Loaded with Silver Nanoparticles: Synthesized via Chinese Holly Plant Leaves

Naseeb Ullah¹, Sohail Yasin², Zamir Abro², Lin Liu², Qufu Wei^{1,*}

¹Key Laboratory of Eco-Textiles, Ministry of Education China, Jiangnan University, Wuxi, Jiangsu, 214122, China

²Key Laboratory of Advanced Materials and Textiles, Ministry of China, Zhejiang Sci-Tech University, Hangzhou, Zhejiang 310018, China

Both Naseeb Ullah and Sohail Yasin contribute equally to this work.

Abstract In this work we investigated cost effective and environment friendly antibacterial properties of cotton fibers loaded with silver nanoparticles (AgNPs) synthesized from natural Chinese Holly plant extracts as a reducing and capping agent. The formation of AgNPs from Chinese Holly plant leaves extract was observed by UV-vis spectrophotometer and was found to be <100nm in size confirmed by electron microscopy. The antimicrobial properties of cotton fibers loaded with silver nanoparticles was evaluated against gram-negative *Escherichia coli* (E.coli) bacteria. The results showed great antibacterial properties by merging 1.5-4.5% of Chinese Holly leave extracts. These cotton fibers also showed fine antibacterial efficiency after several washings making it suitable for medical applications with an ease.

Keywords Nanoparticles, Cotton fibers, Antibacterial properties, Chinese holly leaves

1. Introduction

Due to the recent development in novel technologies regarding synthesis of metal nanoparticles has gained popularity, research related to nanoparticles is expanding because of their vast applications. Usually, nanoparticles are prepared by different chemical methods which are mostly not eco-friendly. This study reports a fast and suitable method of producing silver nanoparticles from Chinese Holly plant leaves and silver nitrate respectively, focusing economical approach for synthesis of AgNPs. As cotton is cellulosic in nature, it makes it more prone towards bacterial growth. So, the cotton fibers are treated with different chemicals to get antibacterial cotton textiles [1-2]. Synthetic methods for the preparation of silver nanoparticles, mostly chemical reducing agents are linked with environmental toxicity or health hazards. Therefore, new development in synthesis of silver nanoparticles from natural extracts is considered to be the most appropriate method keeping the environmental issues accounted [3-5]. Though green synthesis of nanoparticles using plant extracts such as Alfalfa, Aloe Vera, Cinnamomum camphora, Neem, Emblica officianalis, Lemongrass, and Marri has been accounted in recent researches, but the

complete potential of the plants used as reducing agents for the synthesis of nanoparticles is yet to be discovered [6-12].

This study engages with the integration of silver nanoparticles on cotton fibers synthesized via Chinese Holly leaves extract. The cotton fibers having silver nanoparticles also attained great mechanical properties as well, which is due to the functional groups present in extracts of Chinese Holly leaves. Chinese Holly plant (*Ilex Cornuta*) is a species of the genus *Ilex* in Aquifoliaceae plant family. It is native to and abundantly found in Southern China. The Chinese Holly (*Ilex Cornuta*) is valued in horticulture for its attractive and distinctive glance with red berries. The Chinese Holly plant (*Ilex Cornuta*) is famously used for tonic, contraceptive, febrifuge and for carminative purposes. Particularly, some say it gives strength to backbones and knees; the whole plant made into soup, which is used in fever, joints and lower back pain [13-16].

2. Experimental

2.1. Materials

The *Ilex Cornuta* leaves were collected from their trees available in the Xiasha campus of Zhejiang Sci-Tech University, Hangzhou, China. Silver nitrate (AgNO₃) was purchase from Strem Chemicals, Inc. (USA). *E. coli* (ATCC 25922) and *S. aureus* (ATCC 6538) strains were taken from the College of Life Sciences, Zhejiang Sci-Tech University.

* Corresponding author:

soh.yasin@gmail.com (Qufu Wei)

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Commercial washing powder “Tide” was used for washing durability tests. Cotton fibers with 1cm length were used for antibacterial activities. Distilled water was used throughout the experiments.

2.2. Preparation of the Ilex Cornuta Leaves Extract

The extract solution was prepared by boiling 1.5, 3 and 4.5 grams of leaves in an Erlenmeyer flask with 100ml of distilled water for 10 minutes at 100°C followed by filtration and stored at 4°C.

2.3. Synthesis of Silver Nanoparticles

In usual experiment 5ml of the fresh leaves extract was added to a conical flask containing 5ml of 1mM aqueous AgNO₃ solution at room temperature. The silver ions were reduced to silver nanoparticles within 2-3mins by the Chinese Holly plant leaves extract. The quick conversion of solution color showed the formation of silver nanoparticles by observing the color change from colorless to yellowish-brown color.

2.4. Antibacterial Activity

The antibacterial activity against *E. coli* and *S. aureus* was evaluated as an index after 8h of bacterial culture, which was calculated as the percent of bacterial reduction using the following equation;

$$P (\%) = [(C_0 - C) / C_0] \times 100 \quad (1)$$

Where, P is the percentage of bacterial reduction, C₀ is the number of bacterial colonies on the untreated cotton fabrics, and C is the number of bacterial colonies on the AgNPs deposited cotton fibers [17].

The antibacterial activity of cotton fibers loaded with AgNPs was evaluated by using gram-negative bacterium *E. coli* (ATCC-25922) and gram-positive bacterium *S. aureus* (ATCC-6538). The mentioned bacterial strains were inoculated in the sterilized Luria-Bertani (LB) medium and incubated at 37°C overnight with continuous shaking. For qualitative measurement of antibacterial activity, the AgNPs deposited fibers were cut into 1 cm long and the antibacterial activity was tested using a modified agar diffusion assay. The samples were placed on the *E. coli* or *S. aureus* grown LB agar plate and incubated overnight at 37°C followed by the measurement of the inhibition zone against above two microbial species.

Briefly, 15mg of cotton fibers were placed in the sterilized testing tubes and inoculated with 90 to 95 colony forming units (CFU) of bacterial suspension, which were then incubated at 37°C with vigorous shaking in order to assure the sufficient contact between the bacteria and the fabrics. At 4 to 8h contact times, the number of surviving colonies was determined by plating serial dilution on plate count agar to obtain the overall number of bacteria. The antibacterial activity was calculated in accordance with equation (1).

2.5. Washing Durability Test

The laundering durability of antibacterial activities was evaluated using the AgNPs deposited cotton fibers prepared for 30 minutes were washed in the bath containing 0.5% “Tide” washing powder at liquor-to-fiber ratio of 300:1. After 30 minutes of washing at 40°C, the fibers were rinsed at temperature and dried at 37°C. The antibacterial activity of cotton fibers after 0, 2, 3, and 5 washing cycles was determined according to the standard method (dynamic shake flask method) (ASTM-E 2149: 2001, Standard test method for determining the antibacterial activity of immobilized antibacterial agents under dynamic contact conditions).

2.6. Mechanical Properties

The mechanical properties cotton fibers with and without silver nanoparticles were verified by using INSTRON 3369 Universal Testing Machine using 10kg load cell. The cotton fibers with cm length were tested three times and the average values were taken.

3. Results and Discussion

Generally, the methods followed to reduce silver ions to silver nanoparticles, such as; Ultra Violet or Gamma radiation, photochemical, ultrasound or other chemicals are either very expensive or chemically toxic to environment somehow. Therefore, the main purpose of this research was to produce silver nanoparticles without any harmful chemicals relatively using just natural eco-friendly leaves.

3.1. Characterization of AgNPs

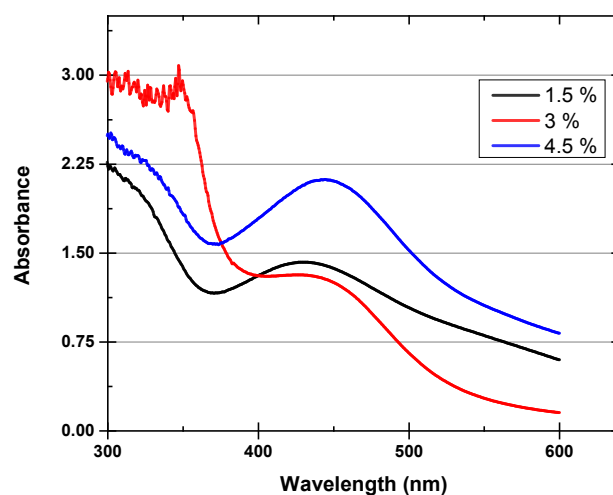


Figure 1. UV-VIS absorption spectra of AgNPs synthesized from 1mM silver nitrate using 1.5, 3 and 4.5% of Chinese Holly leaves extract

After the reaction of AgNO₃ with Chinese Holly leaves extract, it was observed that the color of the solution was changed to yellowish brown due to excitation of surface Plasmon vibrations in the metal nanoparticles. It was observed in UV-visible spectrum (Fig. 1) that surface Plasmon resonance occurred in between 400-450nm

indicating the clear formation of AgNPs. The conversion of Ag⁺ ions into silver nanoparticles was due to the reduction action of functional groups such as flavonoids present in Chinese Holly leaves (*Ilex Cornuta*), different types of flavonoids like; quercetin, hyperin, 3'-methoxydaidzin, isorhamnetin, formononetin, kaempferol etc. [18]. These flavonoids are essential for the reduction process of silver nanoparticles, further, the high molecular chains present in Chinese Holly leaves provide stabilization for the produced silver nanoparticles [19]. On UV-visible spectrum scale, absorption peaks at 438.41, 440.53 and 443.11nm were observed in 1.5%, 3% and 4.5%, respectively which is due to the increase in the concentration of Chinese Holly leaves.

The silver nanoparticles reduced by leaves extract had spherical size with variable diameters which was imaged by high resolution TEM (Fig. 2). Transmission electron micrographs were engaged to foresee the size and shape of the silver nanoparticles formed during the reaction of AgNO₃ and Chinese Holly leave extracts. The images (Fig. 2 a, b and c), were the demonstration of the silver nanoparticles with particles formed in different shapes (spherical - small and large) and sizes, ranging from 7 to 20nm in diameter.

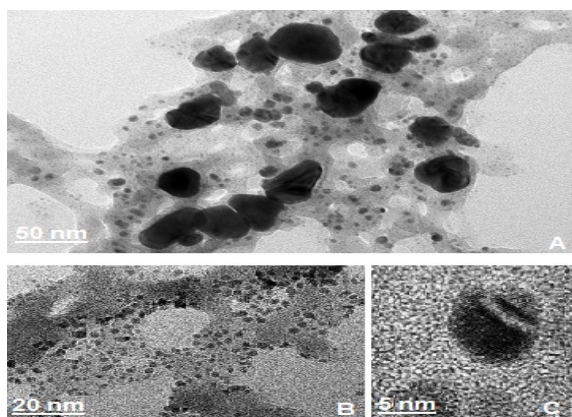


Figure 2. TEM images of the silver nanoparticles synthesized by Chinese Holly plant leave extract showing different particle sizes. (a) 50nm scale, (b) 20nm scale, (c) 5nm scale

3.2. Description of AgNPs Loaded on Fibers

The loading of silver nanoparticles on cotton fibers were carried out by adding silver nitrate solution in leaves extracts broth, after instant reduction process the nanoparticles were stabilized by the polysaccharides. The cotton fibers were submerged into the stabilized silver nanoparticles solution for 24 hours. The silver nanoparticles settled on cotton fibers by polysaccharide or by some other functional groups present in the China Holly plant leaves extracts.

To visually verify the formation of AgNPs on the cotton fibres, cotton fibres along with AgNPs loaded fibres were scanned under scanning electron microscopy (Fig. 3). The SEM images of control cotton fibres with AgNPs loaded fibers exhibits uniform neat plain spun structure and presence of AgNPs on the overall fibres at different scale levels.

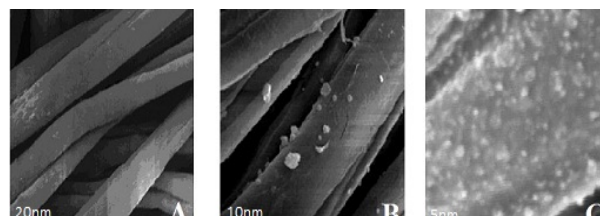


Figure 3. Scanning Electron Microscope images of silver nanoparticles loaded on cotton fibers by using 4.5% Chinese Holly plant leaves extracts. (A) Fibers without AgNPs (B) and (C) Fibers loaded with AgNPs

The loading of silver nanoparticles on cotton fiber was developed by shaker to inhabit the silver nanoparticles on cotton fabrics. The hydroxyl groups of leaves extracts (polysaccharides) stabilized the nanoparticles on the cotton fibers, as cotton fiber consists of cellulose with 1, 4-d-glucosepyranose as its repeating chain units. Cellulosic fibers have extensive surface area meaning cotton fiber can be used to assist the adsorption of silver nanoparticles pretty efficiently on cotton fibers due to hydroxyl group [20].

3.3. Antibacterial Activity

The antibacterial test was carried out by following the Standard SNV 195920-1992, stating a growth inhibition area of sample observed close to be >1 mm in size, the antibacterial properties are marked as 'good'. And if the sample is totally rehabilitated by the bacteria, the antibacterial property is marked as 'not sufficient'. Different levels of antibacterial capacity are related to the dimension of the growth inhibition area around the samples [21]. The antibacterial activity of silver nanoparticles loaded cotton fibers was examined by measuring inhibition zone diameter by digital calliper (Table. 1). In present investigation the cotton fibers loaded with silver nanoparticles developed from Chinese Holly plant leaves have exhibited >1.5mm inhibition zone nearly in all the cases. All title and author details must be in single-column format and must be centered.

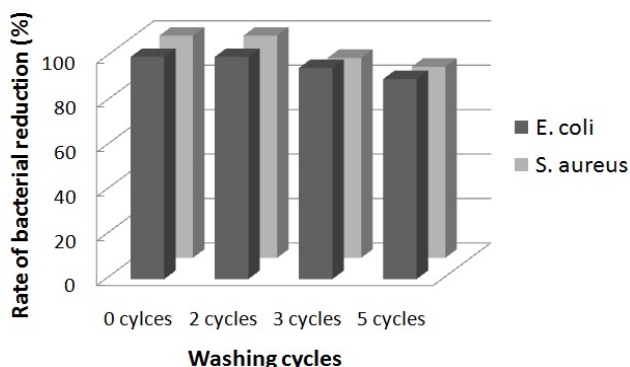
Table 1. Zone diameter interpretation of cotton fibers loaded with silver nanoparticles

AgNPs	Extracts on cotton fibers	Zone diameter without wash (mm)	Zone diameter after 5 washes (mm)
1mg/disc	1.5%	1.5	1.3
	3%	1.6	1.2
	4.5%	1.8	1.5

The cotton fibers treated for 30 minutes were subjected to the repeated washing for the findings of laundering durability of antibacterial property of AgNPs by cultivating the *E. coli* and *S. aureus* strains in LB medium for 18 hours. Figure 4 shows that, the antibacterial properties of cotton fibers after 3 washing cycles were similar to the unwashed fibers. After 5 washing cycles, the antibacterial activity was more than 95%, signifying the exceptional laundry durability of AgNPs loaded fibers.

Table 2. Mechanical properties of cotton fibers treated with Chinese Holly leaves extracts and AgNPs loaded cotton fibers

Samples	Elongation % at breakage		Young's modulus (MPa)		Max. stress (MPa)	
	Extracts	AgNPs depositions	Extracts	AgNPs depositions	Extracts	AgNPs depositions
1.5%	21.82	15.95	291.75	351.58	35.12	30.71
3%	19.37	14.05	270.95	320.21	33.25	28.82
4.5%	18.92	13.37	269.52	309.98	31.68	27.05

**Figure 4.** Laundry durability of AgNPs loaded cotton fibers against *E. coli* and *S. aureus*

The mechanical properties of cotton fibers such as elongation percentage at break, Young's modulus and maximum stress, customized with AgNPs and treated with leaves extract only as well. The elongation percentage at break for the AgNPs loaded cotton fibers was found to be higher than the control fibers and fibers treated with leaves extracts. It is possibly due to the presence of hydroxyl (-OH) groups of chain in cellulosic cotton fibers. The higher values of Young's modulus and maximum stress at load for control fibers was found than the fibers treated with leaves extracts and AgNPs loaded cotton fibers. Table 2 indicates that the AgNPs entrenched on cotton fibers uphold good mechanical properties stating their ability as useful for wound dressings.

4. Conclusions

In the field of nanotechnology development of an environment friendly and toxic free process for the synthesis of silver nanoparticles is vital. This study proudly defines a low cost and organic process for producing nanoparticles by using Chinese Holly plant leave extracts as a reducing agent. The results indicated that silver nanoparticles loaded on cotton fibers by using 4.5% Chinese Holly plant leaves extracts exhibited greater reduction of *E. coli* growth. This is because of the formation of smaller size nanoparticles, as well as a fast release of AgNPs into the medium. Based on this study it is possible to use these cotton fibers for medical applications such as surgical clothes, wound dressing, antibacterial finishing and other textiles.

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