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Herbal Extract-Induced Silver Nanoparticles for Antibacterial Cotton Fabric

Sanjeev Kumar Rajput[1]*, Mukesh Kumar Singh[1], Dinesh Babu Shakyawar[2]

[1] *Uttar Pradesh Textile Technology Institute, Shouterganj Kanpur 208001*
[2] *National Institute of Natural Fibre Engineering and Technology, Kolkata*
* *Corresponding author. E-mail: mr.sanjeevrajput@rediffmail.com*

Abstract
Herbal extract-induced metallic nanoparticles have replaced the traditionally synthesized nanoparticles to achieve sustainability in antimicrobial textiles. Silver nanoparticles (NPs) were created by the bio-reducion of silver nitrate with eucalyptus corymbia leaf extract. The bi-lateral activities of herbal extract, like the reduction and capping of silver nanoparticles, have added new dimensions in the bactericidal sector. Silver nanoparticles were characterized by UV-visible spectroscopy, a particle size analyzer, Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), energy dispersive X-ray (EDX) and X-ray diffractometry. In this study these biosynthesized nanoparticles were applied on cotton fabric alone and along with chitosan by the pad-dry-cure method to create antibacterial clothing. Antibacterial efficiency was characterized using the colony counting method (serial dilution method). The treated fabric shows excellent antibacterial efficiency.

Keywords
Biosynthesis, nanoparticles, natural dye, antibacterial property.

1. Introduction

Nanotechnology has been one of the most emerging and active areas of research in the last two decades [1]. It is a science that deals with materials in the range of 1 to 100 nm [2, 3]. Nanoscience is adding new dimensions to research everyday [4]. Moreover, the synthesis of nanoparticles is increasing exponentially because of its wide range of applications in the field of optoelectronics [5, 6], biosensors [7], bio-nanotechnology [8] and bio-medicine [9].

The biosynthesis of nanoparticles employing biological microorganisms, such as fungi [10] and bacteria [11] or plant extracts [12-14], has been developed as a simple and viable method in comparison to more complex chemical methods. Different nanomaterials such as copper, zinc, titanium [15], alginate [16], magnesium, gold [17] and silver have been developed for different textile applications. Silver nanoparticles have been recognized as the most effective antimicrobial agent against various microbes, such as bacteria, viruses, and fungi [18]. Silver NPs play a vital role in nanomedicine and nanotechnology [19]. The impact of silver nanoparticles on the colour depth and tensile properties of wool and silk fabrics was critically discussed by Chattopadhyaya and Patel [20].

Previous studies also used typical chemicals to synthesize Zn, Au, Ag, Pd, and Pt nanoparticles. The application of toxic chemicals and their drainage after the application is a significant hurdle to establish an environment of sustainable development. Therefore, to achieve the long-term goal of sustainable development, this research work was planned to explore the potential of natural plant sources to produce herbal synthesized nanoparticles in place of toxic chemicals. Eucalyptus corymbia leaf extract was used to bio-reduce silver into nanoparticles. The bio-reduced silver nanoparticles were also applied by the pad-dry-cure method on cotton textile material to enhance the sustainability of the process in the future. The efficacy of eucalyptus corymbia as a reducing agent of silver was characterized. The antimicrobial potential of treated cotton fabric was assessed against *E. coli*, a gram-negative bacteria and *S. aureus*, a gram positive bacteria.

2. Materials & Methods

Fabric - 100% cotton woven fabric with the following specifications - Ends per dm: 425, Picks per dm: 362, and GSM: 125 was procured from a local market, scoured at 80°C in the presence of 1% non-ionic detergent for 30 minutes, hot washed, and air dried.

2.1. Chemicals and auxiliaries

Silver nitrate crystal [Mw. 169.87] was obtained from S.D. Fine Chemicals ltd. Bacterial cultures of E. coli and S. aureus were procured from NCIM Pune. Chitosan was procured from SRL chemicals in Kanpur. Eucalyptus corymbia leaves were collected locally.

2.2. Synthesis of herbal nanoparticles

Twenty grams of dried leaves of eucalyptus corymbia were soaked in 200 ml of distilled water, followed by boiling for 30 minutes, and finally filtered twice to get the desired extract. 0.16987 g of $AgNO_3$ salt was dissolved in 1000 ml distilled water to get a 1mM solution. The herbal extract was added to $AgNO_3$ solution at pH 9 to get a 1:4 ratio (herbal extract: Ag salt solution) followed by continuous stirring for 24 hrs at 120 rpm.

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Then the solution was centrifuged at 12000 rpm in a REMI PR-24 centrifuge. The sediment was washed two times with distilled water and again centrifuged each time. The nanoparticles obtained were dried in an oven at 60°C, from which we got dried nanopowder, which was further characterized.

2.3. Cotton fabric treatment with herbal synthesized silver nanoparticles (H-AgNPs)

The nanoparticles [H-AgNPs] were applied to scoured cotton fabric. Before applying H-AgNPs on the cotton fabric, the fabric was scoured again. These biosynthesized nanoparticles were applied to the cotton fabric by the pad-dry-cure technique, followed by curing at 120°C for 3 minutes.

2.4. Characterization

A Horiba Particle Size analyzer (ZX100) was used to analyse the particle size distribution, where distilled and deionized water was used as a dispersing agent working on the principle of dynamic light scattering. UV-visible spectra of H-AgNPs were recorded using Motras Scientific Instruments-UV plus UV Visible spectroscopy. An FTIR instrument was used to characterize the functional group in the nanoparticle solution in the transmittance mode. The spectra of FTIR were recorded at a wavenumber from 500 to 4000 (cm^{-1}). Surface characterization of the nanoparticles was carried out by means of a Field Emission Scanning Electron Microscope NOVA NANOSEM 450 at 10 KV electron voltage. An Energy Dispersive X-ray (EDX) detector was integrated with the X-ray to obtain the chemical composition. A crystallographic study was performed by means of Panalytical Xpert XRD.

The antibacterial efficiency of the treated samples was quantitatively estimated as per the AATCC100 -2004 method. And this was calculated using the following equation:

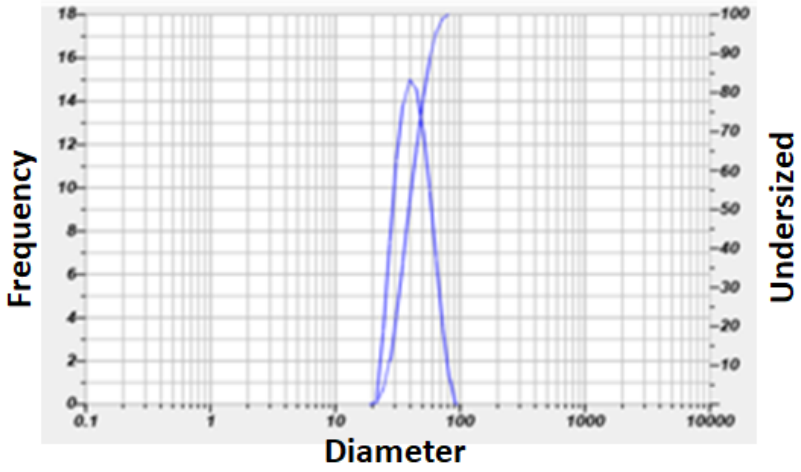


Fig. 1. AgNPs size measurement

$$R = \frac{X - Y}{X} \times 100 \quad (1)$$

Where, R is the treated fabric's bacterial reduction % (antimicrobial efficiency), X (CFUs) is the number of bacteria present in the untreated fabric after inoculation for 24 hours, and Y (CFUs) is the number of bacteria present in the treated fabric after inoculation for 24 hours.

3. Results and discussions

The test results of various characterization techniques are summarized as follows.

3.1. Particle size analysis

Dynamic Light Scattering (DLS) is the most versatile technique used to identify the size of nanoparticles, which is obtained using a Horiba particle size analyzer (nanoParticaSZ-100V2). The nanoparticle analysis suggests that the Z-average value of nanoparticles is 62.7 nm. The particle size distribution follows a Gaussian distribution, as shown in Fig.1. The narrow distribution of AgNPs shows less variability and a uniform size. It also validated the effective reduction potential of Eucalyptus plant leaf extract.

3.2. UV-visible Spectroscopy Analysis

UV- visible spectral analysis is an intense absorption analysis because of the surface plasmon excitation of conduction electrons in metals; silver as metal was used here. In Fig. 2, herbal extract containing Ag-NPs was analyzed in the 290-800 nm range. Silver nanoparticles show a maximum absorption band in the 390-430 nm range due to surface plasmon vibrations of conducting electrons on the silver metal [21, 22].

3.3. FTIR analysis

FTIR analysis of silver nanoparticles containing herbal extract was conducted to prove its role as a reducing agent and capping agent, as well as the presence of several functional groups. In Fig.3, FTIR results are used to establish the presence of functional groups of Eucalyptus corymbia leaf extract responsible for silver particle reduction to Nanoparticles (Ag^{+} to Ag^{0}). The stretching of a bonded hydroxyl group from the phenol and or alcohol group is shown by a band between 3257 and 3382 cm^{-1}, while the stretching of –C=C- stretch from alkenes is confirmed by the absorbance peak at 1652 cm^{-1}, which verifies the presence of alkanols in alkaloids, flavones, and tannins in Eucalyptus corymbia leaf extract [23]. The absorbance at 2123 cm^{-1} confirms the presence of the alkyne group, which has a phyto nature. The

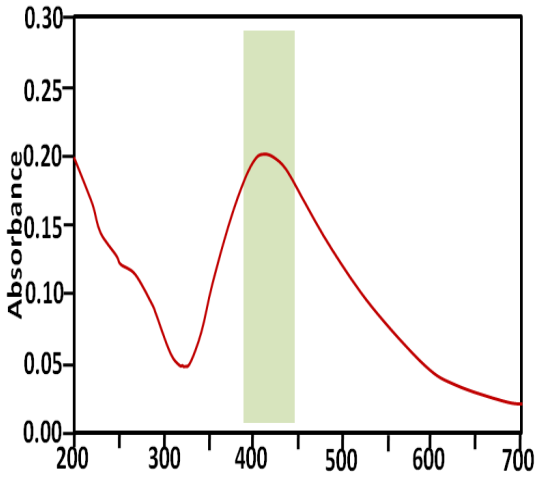


Fig. 2. UV-Visible spectral analysis of nanosolution

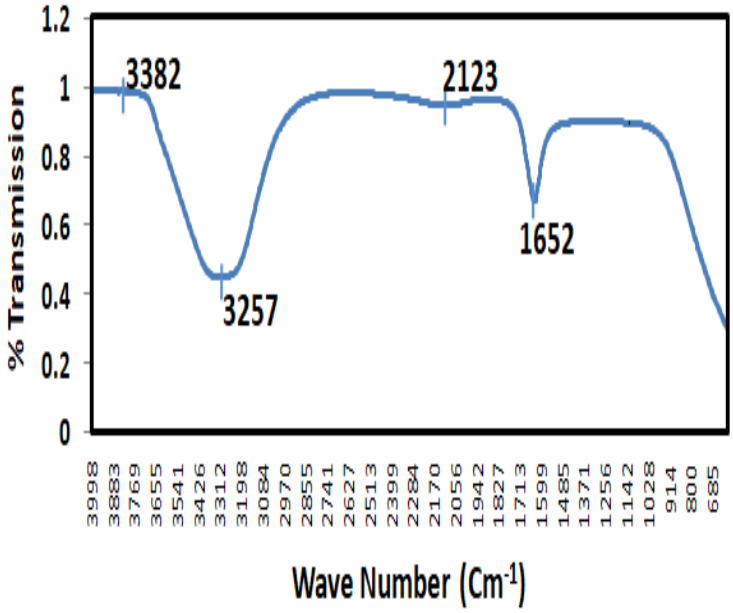


Fig. 3. FTIR analysis of Ag-NPs solution

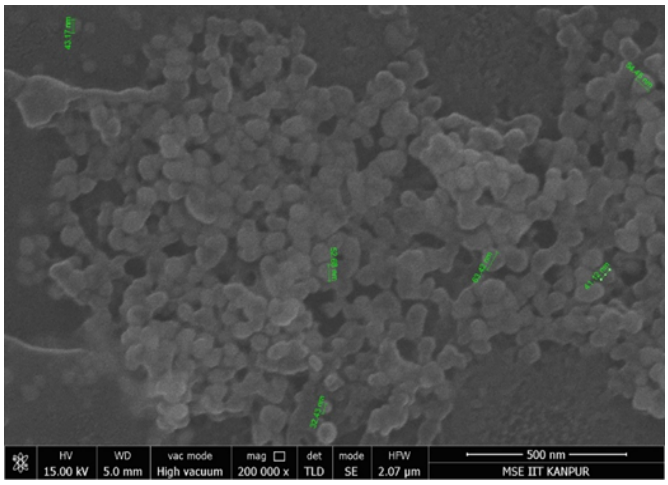


Fig.4. SEM analysis of silver nano particles

FTIR results of this study suggest that the reduction of silver into nanoparticles is guided by (-C=C) and (-OH) hydroxyl groups of Eucalyptus corymbia leaf extract [24, 25].

3.4. FESEM analysis

The aqueous suspension of silver nanoparticles was placed onto clean aluminium foil, allowing the water to evaporate entirely in an oven. Thus, SEM samples of the solution were created.

After that, a specific sample was placed on electrical stubs. Before scanning, all of the samples were gold-coated. FESEM images are shown in Fig 4. The silver nanoparticles are visible, and the particle size distribution is also relatively narrow, which indicates the effective reduction potential of eucalyptus plant extract as an Ag-reducing agent.

It is clear from Fig 4, that the uniformity of Ag-NPs is significantly high. A 62 nm particle size of very high activity was achieved. The particle agglomeration is also visible, confirming these nanoparticles' high reactivity. Maximum particles are found in a dumbbell shape, which again verifies the reduction potential of leaf extract.

3.5. EDX analysis

An energy-dispersive X-ray spectrophotometer exploits the photon nature of light. Because a single photon's energy is only enough to create a quantifiable voltage pulse X-ray in the X-ray range, the output of an ultralow noise preamplifier coupled to the low noise is a statistical estimate of the corresponding quantum energy. A comprehensive representation of the X-ray spectrum is built up practically simultaneously by digitally recording and counting many such pulses in a so-called Multi-Channel Analyzer. The digital quantum counting technique makes the energy dispersive spectrometry technique possible. The EDS approach is quite dependable. The EDX technique can examine microstructures in SEM for their

eZAF Smart Quant Results								
Element	**Weight %**	**Atomic %**	**Net Int.**	**Error %**	**K ratio**	**Z**	**A**	**F**
AlK	36.46	69.64	762.37	4.12	0.2184	0.7175	0.8302	1.0054
AgL	63.54	30.36	419.26	3.71	0.3753	0.5538	1.0672	0.9995

Table 1. Composition of the sample shown by EDX analysis

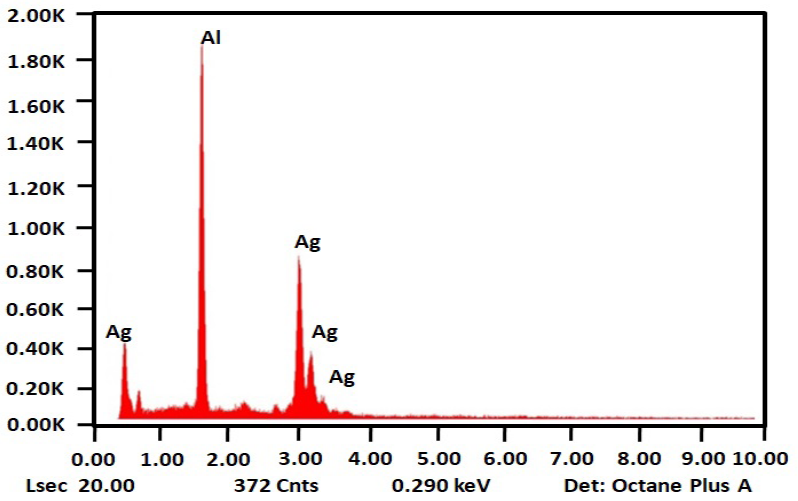


Fig. 5. EDX Analysis of nano particles on aluminum foil

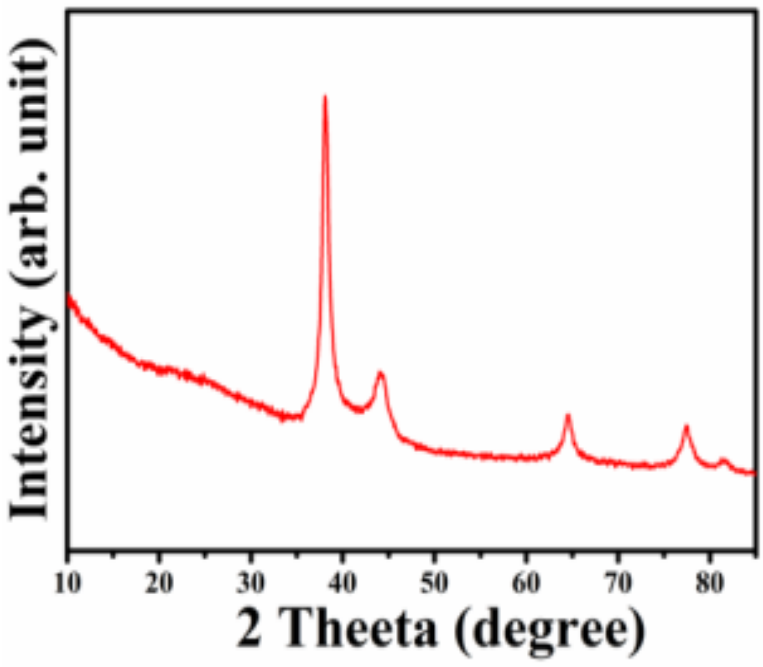


Fig. 6. Crystalline behavior of Ag NPs

elemental composition in greater depth. The elements and their concentrations are calculated reasonably and accurately because this is a non-destructive study. In an SEM, EDS (EDX) determines the material's elemental composition.

In Fig. 5 the EDX data indicate the presence of silver nanoparticles. The nanoparticles were kept on aluminium foil during characterization by EDX. The weight % of silver nanoparticles (AgL) was found to be 63.54% with an atomic share of 30.36%, as shown in Table 1.

3.6. X-ray Diffraction analysis

The X-ray diffraction data ignored the presence of any impurity among the silver nanoparticles, which proves the reduction potential of natural plant Eucalyptus extract.

The full width at half-maximum (FWHM) data was used in Debye-Scherrer's equation (2) to estimate the crystalline dimension of the silver nanoparticles.

$$D = \frac{0.9\,\lambda}{\beta\,\cos\theta} \qquad (2)$$

Where D represents the nanoparticle's mean diameter, λ the wavelength of X-ray radiation, and β denotes the full angular width at half-maximum of the XRD peak at the diffraction angle θ. In Fig. 6, the X-ray diffraction of silver nanoparticles shows prominent peaks at 38.12, 44.26, 64.42, and 77.39 degrees.

These peaks indicate the (111), (200), (220), and (311) planes of silver nanoparticles. The XRD pattern of the nanoparticles fits the face-centred cubic (FCC) crystal structure as given in the X-pert X-ray library file JCPDS no. 89-3722. It is mentioned in Table 2 that various planes of silver nanoparticles have different sizes, indicating that the particles do not have a spherical shape. Various planes have different sizes in nanometers. Plane 111 has an average crystalline size (diameter equivalent) of 11.42 nm, plane 200 - 6.82 nm, 220 - 10.65 nm, and plane 311 - 10.53 nm. These planes showed a lattice constant of 4.10 Å and a cell volume of 68.92 Å, as similarly observed by some other researchers [26,27, 28].

3.7. Antimicrobial potential

The antimicrobial efficiency of all samples was evaluated using the AATCC 100-2004 standard against *E. coli*, a gram-

negative bacteria, and *S. aureus*, a gram positive bacteria. The following equation was used to calculate the percentage of microbial reduction R:

$$R = \frac{X - Y}{X} \times 100 \quad (3)$$

where X (CFUs) is the number of microbiological colonies on untreated fabrics after 24 hours of inoculation, while Y (CFUs) is the number of microbial colonies on treated fabrics after 24 h of inoculation. The antimicrobial potential of fabrics treated with silver nanoparticle and with silver nanoparticles along with chitosan is given in Table 3.

Antimicrobial reduction against *E. coli*, a gram-negative bacteria, is shown in Fig. 7. A sample treated with 100 ppm nanoparticles reduced the bacterial growth by 67%, which then was found to be 38.5% after ten launderings. The silver nanoparticle concentration on the treated samples was increased to 300 ppm; and the bacteria reduction reached 84%, which remained active to reduce bacterial growth by up to 45.2% after ten launderings. The antimicrobial recipe was modified by adding 0.5% and 1% chitosan. The bacterial reduction was enhanced from 82.5% to 93.2% with 0.5% chitosan and silver nanoparticles with 100, 200, and 300 ppm. The antimicrobial potential was reduced by 71.2% to 82.4% after ten launderings, respectively, which indicates the remarkable durability of silver nanoparticles on the cotton fabric surface.

It can safely be said that chitosan works as a coupling agent between cotton cellulose and silver nanoparticles, as shown in Fig.8.

The cotton fabric was first treated with chitosan to provide an active site for the adsorption of silver nanoparticles to be coupled with the cotton fabric surface, which has plenty of hydroxyl groups due to the availability of cellulose in cotton fibre as similarly explained by Xu et al., (2019). The herbal extract-capped silver nanoparticles are coupled with the hydroxyl group present in the cellobiose unit of the cotton fibre, as shown in Fig. 8.

As the chitosan content increased from 0.5% to 1%, the antimicrobial potential of silver nanoparticle treated cotton fabrics was further enhanced by 91.8, 94.6, and 97.8% in the case of 100, 200, and 300 ppm silver nanoparticles.

Figure 9 shows that as the antimicrobial efficacy was measured against *S. aureus*, gram-positive bacteria, the bacterial reduction percentage was quite close to *E. coli* bacterial reduction data. As the samples were treated with 100, 200, and 300 ppm silver nanoparticles, bacterial reduction was found to be 64.2, 72.4, and 79.6%, respectively, and after the

'2θ' of Intense peak	Plane (h, k, l)	Crystalline size (D) nm	Lattice constant (Å)	Cell volume (Å3)
38.12	111	11.42	4.10	68.57
44.26	200	6.82	4.10	69.42
64.42	220	10.65	4.10	68.92
77.39	311	10.53	4.10	68.76

Table 2. Variation in crystalline size, lattice parameter, and cell volume of biosynthesized NPs

S. No.	Sam ple Code	Samples	Bacterial reduction in % (E. coli)	Antibacterial efficiency after 10th laundry	Bacterial reduction in % (S. aureus)	Antibacterial efficiency after 10th laundry in %
1.	S1	Blank sample/untreated fabric	-Nil-	-Nil -	-Nil-	-Nil -
2.	S2	Sample treated only with AgNPs-100 ppm	67.0%	38.5%	64.2	35.0
3.	S3	Sample treated only with AgNPs-200 ppm	76.2%	42.3%	72.4	38.2
4.	S4	Sample treated only with AgNPs-300 ppm	84.0%	45.2%	79.6	42.6
5.	S5	Sample treated with-[chitosan 0.5% + AgNPs-100 ppm]	82.5%	71.2%	78.0	70.8
6.	S6	Sample treated with-[chitosan 0.5% + AgNPs-200 ppm]	87.2%	76.5%	81.3	75.4
7.	S7	Sample treated with-[chitosan 0.5% + AgNPs-300 ppm]	93.2%	82.4%	87.0	81.3
8.	S8	Sample treated with-[chitosan 1% + AgNPs-100 ppm]	91.8%	80.1%	88.1	80.0
9.	S9	Sample treated with-[chitosan 1% + AgNPs-200 ppm]	94.6%	84.2%	91.2	83.7
10.	S10	Sample treated with-[chitosan 1% + AgNPs-300 ppm]	97.8%	90.6%	94.8	89.5

Table 3. Antibacterial efficiency of treated samples

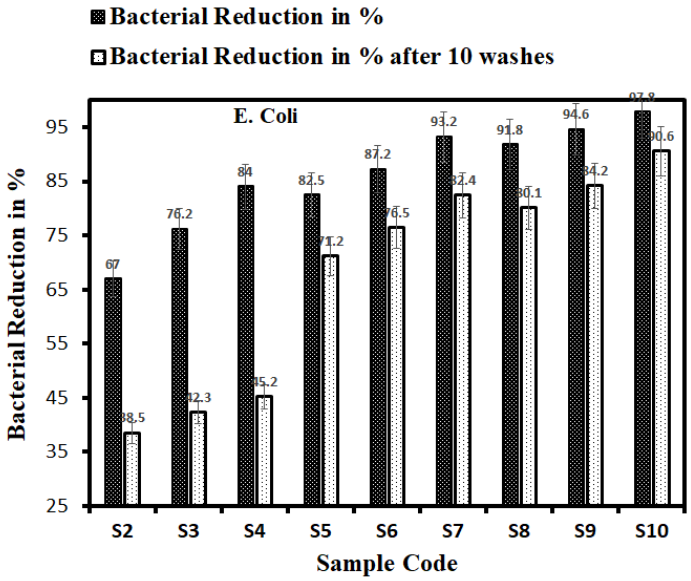


Fig. 7. E. coli bacterial reduction % without washing and after 10 washes

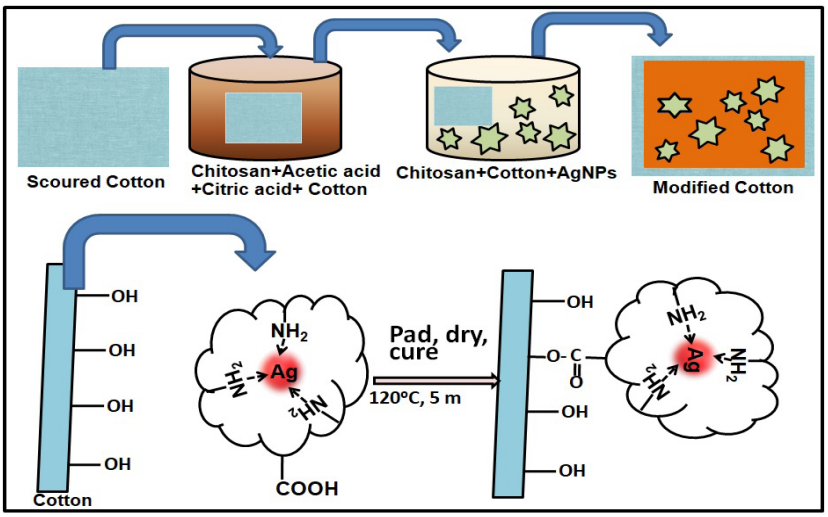


Fig. 8. Role of chitosan in AgNPs adhesion on cotton fabric surface

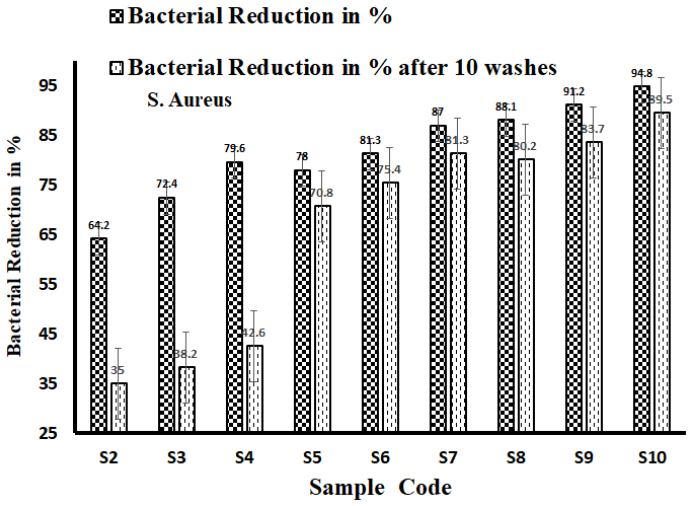


Fig. 9. S. aureus bacterial reduction % without washing and after 10 washes

10th laundry wash, it remained at 35.0, 38.2, and 42.6 %, respectively. When this treatment was done with 0.5% chitosan, bacterial reduction was found to be 78.0, 81.3, and 87.0%, respectively, and it remained at 70.8, 75.4, and 81.3% after the 10th laundry wash. Again when this treatment (with 100, 200, and 300 ppm AgNPs) was done along with 1% chitosan, the bacterial reduction was found to be 88.1, 91.2, and 94.8%, respectively, and remained at 80.0, 83.7, and 89.5% after the 10th laundry wash.

4. Conclusion

- This study concludes that green synthesis of the silver nanoparticle is the safer way of silver reduction.
- Protocol for a cost-effective, efficient and eco-friendly reduction of silver has been developed here.
- The synthesis process is rapid, and the stability of the synthesized nanoparticles is also excellent.
- No stabilizer was used here for the capping of the nanoparticles.
- Eucalyptus leaf extract proved its silver reduction potential up to a nanometre level: of 62 nm, confirmed by a particle size analyzer and FESEM.
- Crystallinity was confirmed by X-ray diffraction analysis and the presence of silver nanoparticles in the sample by EDX analysis.
- The antimicrobial efficacy of 300 ppm AgNPs treated cotton fabric reached 84 % bacterial growth reduction, which was further enhanced to 97.9 % with a 1% addition of chitosan with 300 ppm AgNPs. The antimicrobial potential of green synthesized nanoparticle-treated fabric samples was found entirely satisfactory.
- The durability of fabric treated with AgNPs alone is not so good, but when applied along with chitosan, its durability is satisfactory. This may be attributed to the adhesive nature of chitosan.

Future scope

Green synthesis of nanoparticles is a very economical, eco-friendly, time-saving and sustainable method. Hence, there is good scope to explore new natural resources to synthesize the metal nanoparticles.

There is also sufficient future scope to improve the durability of antimicrobial potential and to enlarge the retention of nano finish on fabric.

Declaration

1. This is original research work and has not been submitted to any other journal for publication in part or as a whole.
2. Authors have no conflict of interest with this research work.

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