

Antimicrobial Effect of Herbal Nanosilver Finished Fabrics on Drug Resistant Pathogens

Margi H. Patel¹, Pratibha B. Desai²

¹Department of Microbiology, Shree Ramkrishna Institute of Computer Education & Applied Sciences, Veer Narmad South Gujarat University, Surat, 395001, India

²Department of Microbiology, Shree Ramkrishna Institute of Computer Education & Applied Sciences, Veer Narmad South Gujarat University, Surat, 395 001, India

Abstract: *Green chemistry plays very significant role in the synthesis of diverse nanomaterials in the field of nanotechnology and nanoscience. Textile materials completely lack protection against microbial growth by its chemical nature. Infestation by microbes cause cross infection by pathogens and develop odour where the fabric is worn next to skin. In addition, staining and loss of the performance properties of textile substrates are results of microbial attack. Basically, with a view to protect the wearer and the textile substrate itself antimicrobial finish is applied to textile materials. Present study focused on green synthesis of silver nanoparticles by silver nitrate and utilizing the bio components of leaves extract of Neem (Azadirachta indica), treatment of Woven & Nonwoven fabrics with nanoparticles, assessment of their antimicrobial efficacy and finally assessment of wash durability. Size of nanoparticles and presence of nanoparticles on the surface of fabrics were confirmed by Scanning Electron Microscope (SEM). Antibacterial activity of silver nanoparticles treated 100% cotton woven fabrics shows maximum 94.06% & 93.65% reduction against Pseudomonas aeruginosa and multi drug resistant (MDR) strain of Pseudomonas aeruginosa and treated SMS Nonwoven fabrics shows maximum 91.01% & 77.27% reduction against multi drug resistant strain of Pseudomonas aeruginosa and Escherichia coli.*

Keywords: Silver nanoparticles, Neem (*Azadirachta indica*) leaves extract, SEM analysis and Antimicrobial activity

1. Introduction

Textiles are one of the most widely used materials in everyday use. The end-use of a textile material dictates its desirable properties. Now a day, consumer's attitude towards hygiene and active lifestyle has led to the incorporation of additional properties to existing fiber types. Chemical treatment of fabrics is one way of adding special properties without hampering the inherent nature of the fabrics. These type of finishes have been developed for application on a wide range of textile materials including treatments for permanent finish, anti-static, anti-UV and anti-microbial etc. Textiles can therefore be designed for special functional uses for applications in defense, firefighters and healthcare environments.

Application of nano scale material and structures are usually ranging from 1-100 nm is an emerging area of nanoscience and nanotechnology. Researchers all around the world are looking for to impart lots of interesting properties such as wrinkle resistance, stain resistance, water repellent, antimicrobial, antistatic etc. by using concept of nanotechnology. Among these properties the ability to make textiles resistant to microbial contamination has an advantage in many applications and market segments especially in medical markets. The spread of infections through textile materials can be controlled by using of antimicrobial textiles that kill pathogens on contact or hinder their ability to reproduce prior to being transferred on to another material or person. It is also pertinent to mention that other than the requirements of the healthcare facilities, increase in consumer's demand for comfort, hygiene and well-being has created a large and rapidly increasing market for antimicrobial textiles [1].

One of the most promising ways to impart antimicrobial property on textile surface is by using metal nanoparticles. Metal nanoparticles have a high specific surface area and unique physicochemical properties. Synthesis of novel metal nanoparticles for the applications such as catalysis, electronics, environmental and biotechnology is an area of constant interest [2]. Silver and its compounds have strong broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times [3]. However, use of silver nanoparticles in textile applications has its own restrictions, as particle size below 50 nm can have a harmful impact on humans and the environment [4, 5]. Several methods such as chemical, physical and biological, have been invented in order to prepare metallic nanoparticles. But, use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol.

Natural herbal products such as Neem [6], Aloe vera [7, 8], Tulsi [9, 10], Green tea [11], Onion skin [12] and Clove oil [8] are exhibit antimicrobial activity. Neem (*Azadirachta indica*), an evergreen tree of India, belongs to the plant family Meliaceae (mahogany). It has been recognized as one of the most promising sources of compounds with insect control, antibacterial and medicinal properties [13]. More than 300 different active compounds have been reported from different parts of Neem tree but, the most important liminoids are azadirachtin, salanin and nimbin. In present study green chemistry route was used to synthesize silver nanoparticles using silver nitrate and bio components of leaves extract of Neem. Finally antimicrobial effect of silver nanoparticles treated fabrics were assessed against multi drug resistant pathogens.

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2. Materials and Methods

2.1. Materials

2.1.1. Media & Chemicals

Muller Hilton Agar (MH Agar) procured from Hi-media was used to assess antimicrobial activity. Nutrient broth was used to make medium for microbial culture, which was used as inoculum. Quaternary ammonium compound (QAC) was purchased from Sigma-Aldrich and silver nitrate (AgNO_3) was obtained from Qualigens.

2.1.2. Microorganism

The strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used. These strains were purchased from MTCC (Microbial Type Culture Collection). The MDR (Multi Drug Resistant) Strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were also used & these strains were obtained from Sterling hospital, Bhavnagar.

2.1.3. Substrate

A fine-medium weight, 100% cotton woven fabric (plain weave, $75 \times 30 \text{ g/m}^2$, ends 75/inch; picks 60/inch) and a polypropylene spunbonded/melt blown/spunbonded (SMS) nonwoven were used for the application purpose.

2.1.4. Medicinal Plant

Herbal medicinal plant Neem (*Azadirachta indica*) was selected for present study. Neem leaves were collected from Sir P.T. Science college Botanical garden located in our college campus at Surat (Figure-1).



Figure 1: Herbal plant: Neem (*Azadirachta indica*)

2.2. Methods

2.2.1. Selection of plant material and preparation of the extract

Plant extract has been prepared from green Neem leaves. Young leaves weighing 5g were thoroughly washed in distilled water. After that leaves were cut into fine pieces and boiled with 100 ml distilled water and filter through whatmann No.1 filter paper. This filtrate was further used for preparation of nanoparticles.

2.2.2. Synthesis of the Silver nanoparticles

Silver ions can be reduced to nanosize by using reducing agent. In this study aqueous extract of Tulsi leaf was used as a reducing agent. 5 ml of plant extract was added into 100

ml of 1mM AgNO_3 solution in 250 ml conical flask at room temperature in dark. After 2 hours, colour change (brown colour change) indicates the reduction of silver ions.

2.2.3. Characterization of synthesized nanoparticles by SEM

Topographical characterization of the nanoparticles were carried out using Scanning Electron Microscope (JEOL-JSAL 6360) at 1000 X and 25,000 X.

2.2.4. Grafting of substrate with nanoparticles, Silver nitrate and Quaternary ammonium compound

Silver nanoparticles, silver nitrate and Quaternary ammonium compound were applied on cotton and SMS fabrics using pad-dry-cure method. Test fabrics were cut into the size of 30 X 30 cm and immersed in the solution containing Silver nanoparticles (2%), 0.01M AgNO_3 solution (2%) and Quaternary ammonium compound (2%) in three separate conical flasks. Citric acid binder (1%) was added in all three flasks and kept for 10 min. Then it was passed through a padding mangle running at a speed of 15 m/min with a pressure of 2 kgf/cm^2 to remove excess solution. A 100% wet pick-up was maintained for all of the treatments. After padding, fabric was air-dried and then cured for 3 min at 140°C . Fabric without any treatment was used as a control.

2.2.5. Antimicrobial assessment of finished fabrics

Test organisms viz. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were used as representative of various groups of bacteria respectively Gram positive, Gram negative enteric and Gram negative bacteria as per AATCC standards. The MDR (Multi Drug Resistant) Strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were also used as test organisms. Qualitative antimicrobial assessment was done using Agar diffusion method (AATCC test method 147) and quantitative antibacterial assessment was done by Percentage Reduction Test (AATCC 100).

2.2.5.1. Agar diffusion test (AATCC 147)

Agar diffusion test is only qualitative, but is simple to perform and is most suitable when a large number of samples are to be screened for the presence of antimicrobial activity. In these tests, first inoculate test organisms in sterile nutrient broth separately and incubated at 37°C for 18-24 h, which was used as inoculum. Test organisms are inoculated on Muller Hilton agar plates over which textile samples are laid for intimate contact. Sample plates are then incubated at 37°C for 18-24 h and examined for growth of bacteria directly underneath the fabrics and immediately around the edges of the fabrics (zone of inhibition). No bacterial growth directly underneath the fabric sample indicates the presence of antimicrobial activity. If antimicrobial agent can diffuse into the agar, a zone of inhibition becomes apparent and its size provides some indication of potency of antimicrobial activity or the release rate of the active agent.

2.2.5.2. Percentage reduction test (AATCC 100)

Non-sterile test specimens were taken, and they were cut into pieces according to standard size (20 mm radius) with round shape recommended by AATCC and sterilized under UV. Test specimens were soaked inside the test tubes

containing sterile Nutrient broth and a loop full of test organisms was inoculated. Initial cell concentration was calculated by using viable count method. Test samples were incubated at 37° C for 18 hours. After 18 hours of incubation, final concentrations of cell in control and test samples were calculated using viable count method. Sample plates were incubated at 37°C for 18-24 hours. After incubation period numbers of colonies present in each dilution plates were counted. % reduction in bacteria was calculated using the following formula

$$\% \text{ Bacterial Reduction} = [(A-B)/A] \times 100$$

A = initial no. of cells; B = final no. of cells

2.2.5.3. Wash durability testing

Wash durability testing of the finished fabrics were carried out using a neutral soap at 40° C (+/- 2° C) for 30 minutes, keeping the M: L ratio at 1: 50, followed by rinsing washing and drying. After drying the test fabrics and the control were assessed for antimicrobial activity by Agar diffusion test (AATCC 147).

2.2.6. Characterization of Silver nanoparticles grafted test fabrics by Scanning Electron Microscopy

Topographical properties of Silver nanoparticle grafted test substrates after treatment and post-laundrying at 10 wash cycles were done using SEM (Scanning Electron Microscopy) at different magnification.

3. Results & Discussion

3.1. Biosynthesis of silver nanoparticles



Figure 2: (A) 1mM AgNO₃ solution without plant extract and (B) 1mM AgNO₃ solution with Neem leaf extract after 2 hours

Figure-2 (A) and (B) are the photographs of AgNO₃ solution without Neem leaf extract and AgNO₃ solution with Neem leaf extract after 2 hours. As shown in Figure-2, Silver nanoparticles (AgNPs) were synthesized using Neem leaf extract under static condition in dark, which was yellowish brown in color. AgNPs were synthesized within 2 hours by using Neem leaf extract.

3.2. Characterization of silver nanoparticles:

Synthesized AgNPs were characterized by SEM as shown in Figure-3. It is seen that spherical shaped AgNPs were obtained in case of Neem leaf extracts being used as reducing agent. SEM analysis of AgNPs grafted medical textiles (100% cotton woven & SMS nonwoven) under study were also showed in Figure-3. SEM analysis of synthesized silver nanoparticles using Neem leaf extract showed particles size in the range of 58-82 nm.

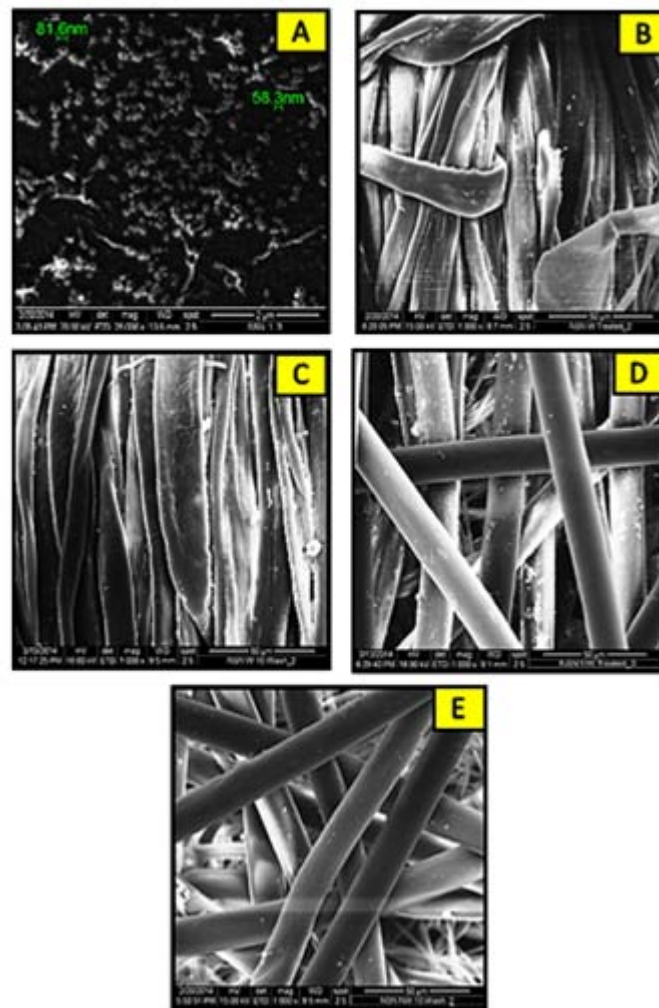


Figure 3: Scanning Electron Microscopic detection of (A) AgNPs synthesized using Neem leaf extract, Synthesized AgNPs treated 100% cotton woven fabric (B) without laundry wash and (C) after 10 laundry wash at 1000 X magnification, Synthesized AgNPs treated SMS nonwoven fabric (D) without laundry wash and (E) after 10 laundry wash at 1000 X magnification.

3.3. Antimicrobial assessment of finished fabrics

3.3.1. Agar Diffusion Test (AATCC 147)

Antimicrobial activity of as treated SMS Nonwoven and 100% cotton woven fabrics were assessed by agar diffusion method. In qualitative test, Figure 4 indicates that all the coated woven fabric exhibit antimicrobial activity based on zone of inhibition for *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* as well as drug resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, but QAC treated 100% cotton woven fabric does not show its antibacterial activity against *Pseudomonas aeruginosa* (MDR). Maximum zone of inhibition was found against *Pseudomonas aeruginosa* and drug resistant strain of *Pseudomonas aeruginosa* with a zone size of 17 mm in case of AgNPs treated fabric. In case of SMS Nonwoven fabric QAC treated fabric did not show its antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* (MDR). Except these SMS Nonwoven fabrics all other treated fabrics show their antibacterial activity against test organisms (Figure 5).

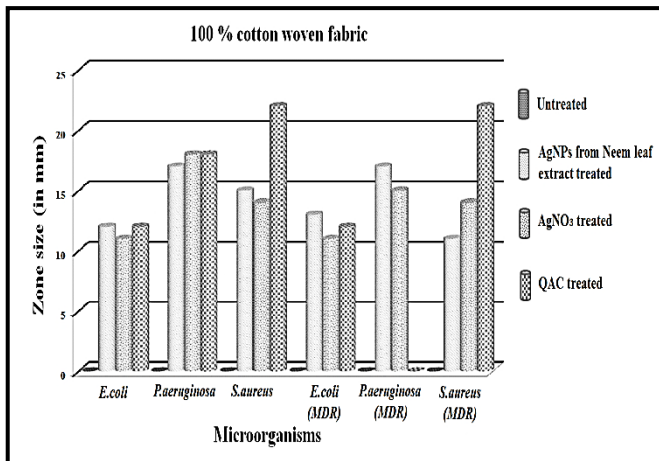


Figure 4: Antimicrobial Assessment of 100% Cotton woven fabric –AATCC 147

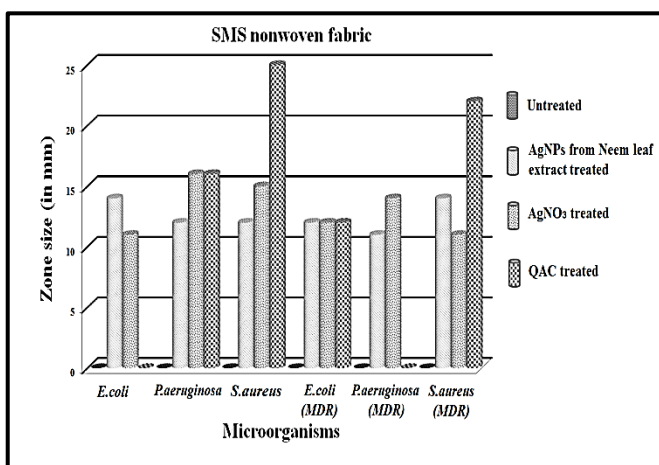


Figure 5: Antimicrobial Assessment of SMS Nonwoven fabric –AATCC 147

In case of AgNPs treated SMS nonwoven fabric maximum antibacterial activity was found against *Escherichia coli* and drug resistant strain of *Staphylococcus aureus* with a zone size of 14 mm.

3.3.2. Percentage Reduction Test (AATCC 100)

Quantitative assessment data for antibacterial activity of AgNPs grafted 100% cotton woven and SMS nonwoven fabrics are shown in Figure 6. In case of AgNPs treated 100% cotton woven fabric, maximum percentage reduction was observed against *Pseudomonas aeruginosa* with a percentage reduction of 94.06%, followed by 93.65% for drug resistant strain of *Pseudomonas aeruginosa* and 92.04% for *Staphylococcus aureus*. AgNPs synthesized from Neem leaf extract treated SMS nonwoven fabric possessed excellent antimicrobial activity against drug resistant strain of *Pseudomonas aeruginosa* with a reduction of 91.01% as shown in Figure 6.

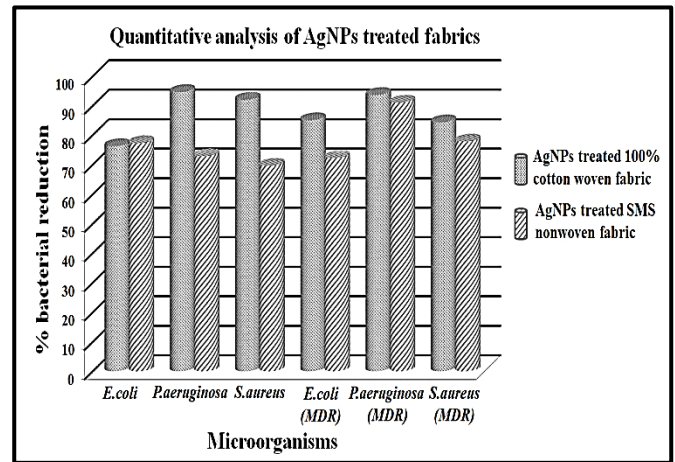


Figure 6: Effect of bacterial count against AgNPs treated 100% cotton woven and SMS nonwoven fabrics

3.3.3. Wash durability test

Comparative durability of AgNPs, AgNO₃ and QAC treated 100% cotton woven and SMS nonwoven fabrics against 10 laundry cycles are illustrated in Figure 7 and 8 respectively against all test microorganisms.

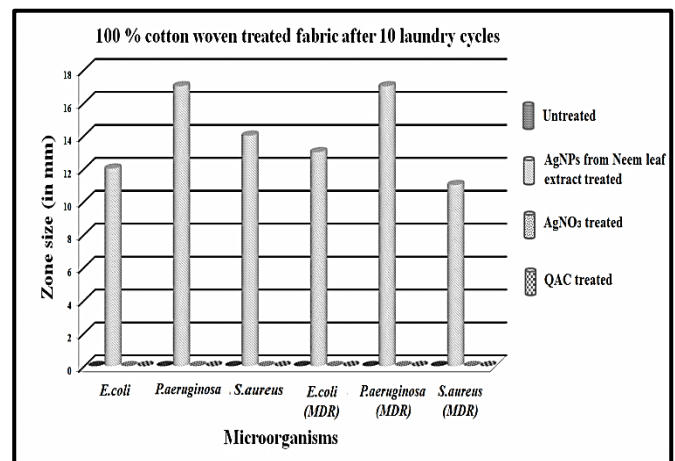


Figure 7: Antimicrobial Assessment of 100% Cotton woven fabrics after 10 wash cycle–AATCC 147

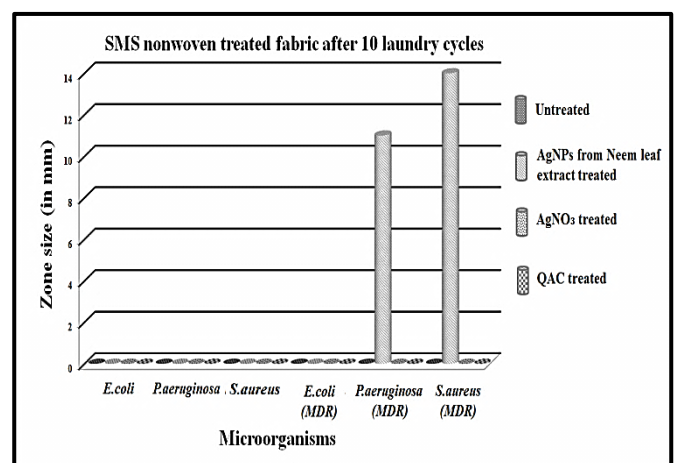


Figure 8: Antimicrobial Assessment of SMS Nonwoven fabrics after 10 wash cycle–AATCC 147

Results are summarized as graphs (Figure 7) support the fact that AgNPs synthesized from Neem leaf extract treated 100% cotton woven fabric have superior durability to

laundering than the QAC and AgNO₃ treated same fabrics. And for AgNPs treated SMS nonwoven fabric has moderate activity against only multi drug resistant strain of *P.aeruginosa* and *S.aureus* after 10 laundry cycles (Figure 8).

One fact about silver nanoparticles is that they exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [6]. On adding aqueous extract of Neem leaves to AgNO₃ solution, colour of reaction medium changed from colourless to yellowish brown [2]. Tripathy et al., [2] had reported synthesis of AgNPs using neem extract within 24 hours but in present study AgNPs were synthesized within 2 hours.

Bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio which allows them to interact closely with microbial membranes. Oxygen associated with silver reacts with sulfhydryl (-S-H) groups on the cell membrane to form R-S-S-R bonds causing inhibition of respiration resulting in cell death [14]. Asmita et al., [14] had reported synthesis of silver nanoparticles using extract of neem leaf and triphala and evaluation of their antimicrobial activities. They had also reported the antimicrobial activity of AgNPs against multi drug resistant strain of *E. coli* was 11 mm zone size. Our findings are in accordance with Asmita et al., 2012.

AgNO₃ is leaching type of antimicrobial agent [15]. In the presence of moisture, this antimicrobial agent is continuous release from the textile into their surroundings, where they act as a poison to a wide spectrum of bacteria and fungi [16]. Due to leaching of AgNO₃ into its surroundings, concentration of the active substance in textile decreases and gradually falls under the limit of effectiveness. This type of effect can induce resistance to this substance in microorganisms and this leaching agent does not withstand repeated laundering [17].

QAC typically possess a silane base at one end of molecule and a long molecular chain of carbon atoms at the other end [18]. QAC are active against a broad spectrum of microorganisms such as Gram-positive and Gram-negative bacteria, fungi and certain types of viruses [19]. Despite of many positive properties, QAC has an inherent weakness: leaching from the textile. There are no reactive functional groups in structure of QAC that allow its chemical bonding to the fibers. Owing to the lack of physical bonding, leaching of QAC occurs, resulting in a fast decrease in concentration to below the MIC (Minimal Inhibition Concentration). In addition, QAC has poor wash durability [17].

Balakumar et al., [20] had reported that molecule with Nano size possess more surface area than the bulk molecule. This property enables nano size molecule to adhere on the fiber surface. So, in present study, this property of AgNPs synthesized from Neem leaf extract enables AgNPs to stick on the 100% cotton fiber surface till 10 laundry cycles. But in case of SMS nonwoven fabric, it has low absorption capacity. Due to this reason, AgNPs cannot penetrate inside the fabric surface and not strongly bound to fiber surface. So, in present study, AgNPs from Neem leaf extract treated SMS nonwoven fabric was not accounted effective

antimicrobial activity against test microorganisms after 10 laundry cycles.

Problems related to durability of treated fabrics with more laundry cycles can be sorted out by using non-ionic binders, cross linkers such as formaldehyde, glyoxal, glutaraldehyde etc. or by doing graft polymerization of monomer containing amino groups onto fabric.

4. Conclusion

Silver nanoparticles of spherical shape and 58-83 nm size were synthesized from green chemistry which is eco-friendly route and inexpensive. Bio-components present within Neem leaf plays an important role in reduction of silver to silver nanoparticles. Further, these synthesized silver nanoparticles shows antibacterial activity on Gram positive and Gram negative bacteria and also multi drug resistant strains of Gram positive and Gram negative bacteria.

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Author Profiles



Dr. Pratibha B. Desai is Director of Shree Ramkrishna Institute of Computer Education & Applied Sciences, Veer Narmad South Gujarat University, Surat, Gujarat (INDIA). She is also a member of Departmental Research Studies Committee for Bioscience Department of Veer Narmad South Gujarat University.



Mrs. Margi H. Patel is pursuing her M.Phil in Microbiology under guidance of Dr. Pratibha B. Desai at Shree Ramkrishna Institute of Computer Education & Applied Sciences, Veer Narmad South Gujarat University, Surat, Gujarat (INDIA).