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Research Article

USE OF HERBAL NANO SILVER FOR FABRICATION OF ANTIMICROBIAL COTTON FABRICS AND TESTING ITS EFFICACY AGAINST MICROBES

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ABSTRACT

The development of antimicrobial cotton fabrics using herbal nanosilver has been investigated in this present work. The herbal nanosilver were synthesized by the herbal plant extract method and were directly applied onto the cotton fabric with the help of pad-dry-cure method. The antimicrobial activity of the finished fabrics was assessed qualitatively by agar diffusion and quantitatively by percentage reduction test. The antifungal activity of the finished fabrics was assessed qualitatively by agar diffusion methods. The topographical nature of the both treated and untreated fabrics was studied. The results show that the finished fabric demonstrated significant antibacterial activity against both bacteria and fungus in both qualitative and quantitative methods. The scanning electron microscopic analysis revealed the embedding of herbal nanosilver in treated fabrics. The study of wash durability of the herbal nanosilver treated fabric was also carried out and found to withstand up to 16 washings. The present investigation finds out the presence of herbal compound in the surface of nanosilver, it shows the good antimicrobial and washable durability.

Keywords: Herbal nano silver, Antimicrobial, Antifungal.

INTRODUCTION

Textiles are interesting materials for applying in several medical applications, including hospital cloths and linens; prosthetic valves; and wound dressings (Petrulyte, 2008). Due to the growing demand for comfortable, hygienic, and germ free textile goods, the vital need for the manufacture of antimicrobial textile fabrics has arisen (Chen and Schluesener, 2008).

In textile cotton fiber, provide greater surface area with an outstanding environment to support the microbial growth. It will frequently lead to intolerable smell, dermal contamination, product deterioration, allergic reaction, and other skin related diseases. There are different types of microorganisms both bacteria and fungi which cause community health concern (Weber and Rutula, 2001).

The wearing of clothing coupled with factors such as contamination of skin by feces, urine, and other body effluents and the provision by garments of moisture and darkness can increase the probable infections (Bajpai *et al*., 2007). Microbial growth increases with increasing moisture and repeated laundering of fabrics, and is maximal at neutral pH values and grows well in darkness (Vigneshwaran, 2006). The cotton fabrics are damaged by microbial infection by secondary wall of cellulosic fabric may be directly damaged by fungal hypha (Payne and Kudner, 1996), bacterial decomposition of cellulose takes place from outside to inside (Freddi et al., 2004), cellulolytic microorganisms secrete enzymes which make cellulose soluble, and degrade the fabrics, carbon heterotopy types of bacteria degrade polysaccharide chains into shorter ones which are eventually hydrolysed to shorter oligomers like cellobiose to D-glucose (Chun and Gamble. 2007).

It was seen that simple laundering failed to eliminate these pathogens. With the initiation of innovative methodologies, the increasing requirements of consumers in terms of fitness and hygiene can be fulfilled without compromising issues related to safety, human health, and the environment (Wasif and Laga, 2009). Therefore, antimicrobial finishing should be necessary features of protective textile materials, especially in some risky situations, such as therapeutic applications.

Most of the processes to create antibacterial fibers entail the attachment of biocidal or bacteriostatic agents to the fabric surface, for example, N-halamine, enzyme, quaternary ammonium salt, Chitosan, or zinc oxide (Liu and Sun, 2006; Obendorf and Sun, 2007; Ibrahim et al., 2007; Son et al., 2006). Because of the developing resistance of bacteria against bactericides and antibodies and the irritant and toxic nature of some antimicrobial agents, nanobiotechnological researchers have paid attention their research on nano-sized metal particles such as Ag, titanium dioxide, and copper. Silver, in its many oxidation states (AgO, Ag^{+}, Ag, and $Ag3^{+}$) has been recognized as an element with a strong biocidal action against many bacterial strains and microorganisms (Lansdown, 2002). *Ocimum sanctum* Linn. is one of the well known and highly valuable herbal plant and its extracts have been used to treat cough, warts and microbial infections (Singh *et al*., 1996; Singh and Majumdar, 1997; Rabelo *et al*., 2003). The most important chemical constituents in *O.sanctum* leaf extract are eugenol, carvacrol, tannins, methyl eugenol and caryophyllene (Anul Hakkim *et al*., 2007; Lukmanul Hakkim *et al*., 2008; Raj *et al*., 2003).

Therefore the present investigation was made for testing the antimicrobial efficiency and its wash durability of nanosilver synthesized from *O.sanctum* leaf extract loaded on cotton fabrics and testing.

MATERIALS AND METHODS

Chemicals

All analytical reagents and media components were purchased from Hi-Media (India).

Synthesis of herbal nano silver

The *O. sanctum* plant leaf broth solution was prepared by taking 20g of leaves, cut into small pieces and ground in a mortar and pestle with 100 ml of sterile distilled water and centrifuged at 3000 RPM to get the leaf extract. Take 25mL of leaf broth in the flask and added to 200 ml of 1mM aqueous $AgNO_3$ solution for reduction of Ag^{+} ions. The flask was kept at room temperature on shaker for 24 hrs.

After the incubation period the herbal nanosilver thus obtained was purified by repeated centrifugation at 15,000 RPM for 20 min followed by re-dispersion of the pellet in deionized water.

Characterization of herbal nanosilver

The presence of herbal nanosilver in the solution was characterized by UV-Vis spectrophotometer, FT-IR spectrophotometer, and scanning electron microscope.

For UV-Vis spectroscopy, the production of herbal nanosilver was monitored by measuring the absorbance of the reaction mixture in a range of wavelength from 200 to 800 nm to find out the plasmon peak different intervals (0 min, 30 min, 60 min, 2 h, 4 h, 8 h, 16 h, and 24 h). For FT-IR spectroscopy, herbal nano silver powder was subjected to FTIR spectroscopy measurement (Perkin Elmer

spectrophotometer) in the reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. For SEM analysis, herbal nanosilver was analyzed under a Scanning Electron Microscopic (JOEL) at a voltage of 120 KV.

Loading herbal nanosilver on cotton fabrics

In order to impregnate cotton fabrics (5 cm × 5 cm size) with herbal nanosilver, these were submersed in an Erlenmeyer flask (50 ml) containing herbal nanosilver solution and shaking at 600 RPM for 24 h along with commercial additives and dried at 700C. The resulting dark brown color of the grafted fabric indicated the loading of herbal nanosilver particles within the cotton network part of the fabric.

Characterization of herbal nanosilver loaded cotton fabrics.

The Fourier transform infrared (FTIR) spectra of herbal nanosilver loaded fabric were recorded with an FTIR-Spectrophotometer using KBr. For this, the fabric was sliced into tiny pieces and mixed with KBr. The scans recorded were and the spectral range was 400 to 4000 cm^{-1}. For SEM analysis, herbal nanosilver loaded cotton fabrics was analyzed under a Scanning Electron Microscopic (JOEL) at a voltage of 120 KV.

Quantitative Antibacterial test

The antibacterial properties of herbal nanosilver treated cotton fabrics were quantitatively evaluated against *E.coli*, *Staphylococcus aureus, Pseudomonus aueroginosa, Micrococcus leutus, Proteus vulgaris* according to an AATCC 100 test method.

The fabric samples with 5.0 ± 0.1 cm in diameter were placed in a 250 ml in screw cap jar and inoculated 1.0 ± 0.1 ml of bacterial inoculums. After incubation over contact phase of 24 hrs, 100 ml of sterilized double distilled water was added into the jar and mixed vigorously for 1 min. The solution was then serial diluted to 10^1, 10^2, 10^3, and 10^4. The diluted solution was spread on a nutrient agar and incubated for 24 hrs at 37 ± 2°C. Colonies of bacteria recovered on the agar plate were counted and the percent reduction of bacteria (R) was calculated by the following equation:

$$R (\%) = (B - A) \times 100 / B \quad (1)$$

The A is the number of bacteria colonies from treated specimen after inoculation over 24 hrs contact period, and B is the number of bacteria colonies from untreated control specimen after inoculation at 0 contact time.

Wash durability of the finished fabric

The wash durability testing of the finished fabrics was carried out using a neutral soap at 40º C (+/- 2º C) for 30 minutes, keeping the material : liquor ratio of 1: 50, followed by rinsing washing and drying (Sarkar *et al*., 2003). After drying the test fabrics and the control were assessed for antimicrobial activity by AATCC 100 test method.

Antibacterial test of Agar diffusion method

Nutrient agar was poured in sterile petri plates and one day old broth cultures of the test organisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aueroginosa, Micrococcus leutus,* and *Proteus vulgaris* were used as inoculums. The sterile cotton swab was used for the test organisms were swabbed over the surface of the nutrient agar plates. The cotton fabrics of both treated herbal silver were lightly pressed in the center of the culture. Then the inoculated plates were incubated at 37°C for 24 hours.

RESULTS

There was a visible color modification after the silver nitrate solution was provided to the those leaf extracts. Initially the leaf extract was green in color. Upon adding the silver nitrate solution, it turned yellowish brown color. The presence of silver nanoparticles was confirmed by obtaining a spectrum in the visible range of 200nm to 600nm. A typical peak at 421.5nm was obtained due to the surface plasmon peak of silver nanoparticles (Fig.1).

FTIR measurements were carried out to identify the probable biomolecules liable for capping and stabilization of the silver nanoparticles synthesized by leaf extract. The herbal nanosilver sample shows peaks at 3313.48, 3193, 2976.90, 2883, 1670, 1452, 1338, 1196.78, and 1112.75cm^{-1} (Fig. 2).

The herbal nanosilver finished cotton fabric samples were observed visually and the topography or morphology of the fabric samples was analyzed using high resolution SEM with suitable accelerating voltage and magnification (1000x). The photographs are shown in Fig. 3. From this figure, it shows that continuous herbal nanosilver has deposited on the finished fabric. The size of herbal nanosilver at about 150 nm can be observed.

The test for the zone of inhibition was carried out on petri plates as shown in the figure 4a- e for bacteria and figures 5a – b for fungi. Totally seven organisms were chosen for present research work. Among them, the highest antimicrobial activity expressed by herbal nanosilver coated cotton fabrics on *Pseudomonas aueroginosa* followed by *Proteus vulgaris*. In fungal organism the antifungal activity of herbal nanosilver coated cotton fabric are little lower than bacteria (Table 1).

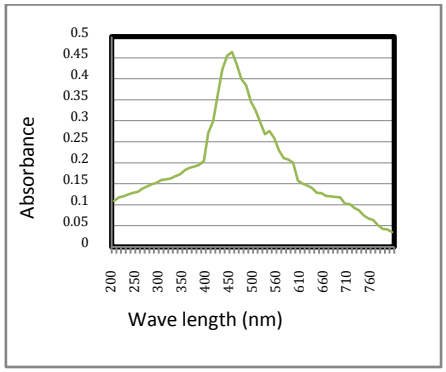


Fig. 1: Plasmon peak herbal nanosilver from absorption spectrum

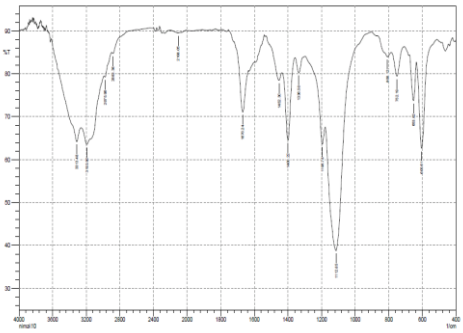


Fig. 2: FTIR spectra of silver nanoparticles synthesized from leaf extract of *O.sanctum*

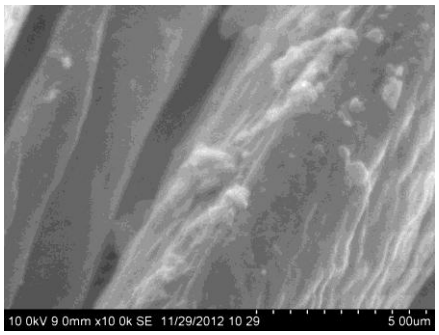
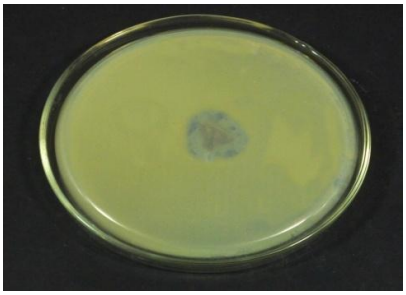
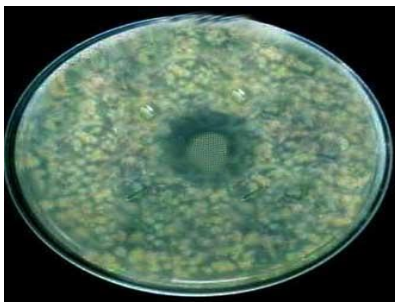


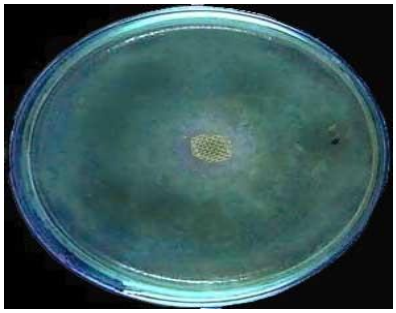
Fig. 3: SEM image of nano silver coated Cotton fabric



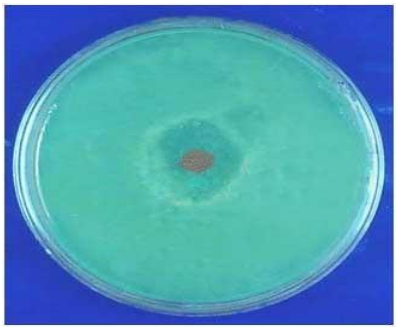
a. S.aureus



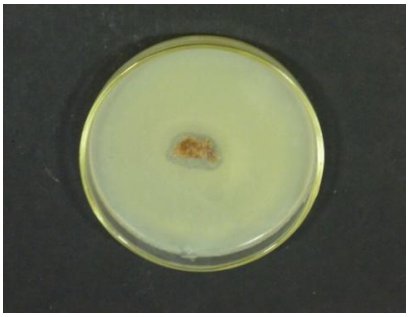
b. Pseudomonas putida



c. Proteus vulgaris

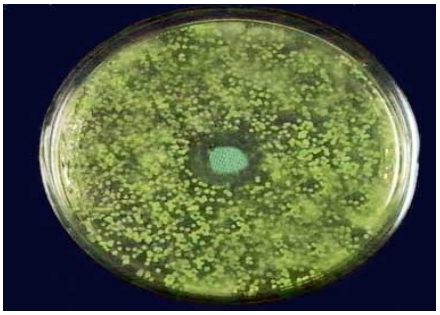


d. Micrococcus leutus

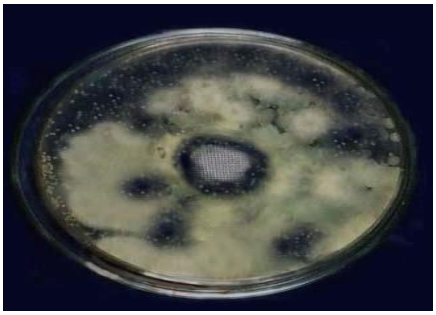


e. E.coli

Fig.4: Testing the antibacterial efficacy of herbal nanosilver loaded cotton fabrics different bacterial organisms



a. Aspergillus niger



b. Candida albicans

Fig. 5: Testing the antifungal efficacy of herbal nanosilver loaded cotton fabrics on different fungal organisms

Table 1: Testing the antimicrobial efficiency of herbal nano silver loaded cotton fabrics by agar diffusion method Zone of Inhibition (in cm)

S. No.	Microbial organism	Cotton with Ag np (mm)
1	*E.coli*	17
2	*S.aureus*	13
3	*Pseudomonus aueroginosa*	28
4	*Micrococcus leutus*	12
5	*Proteus vulgaris*	21
6	*Aspergillus niger*	8
7	*Candida albicans*	6

The quantitative bacterial reduction was studied by percentage reduction test and the results were shown in the Table 2. The results of this percentage reduction test correspond with that of the agar diffusion and parallel streak method. The herbal nanosilver coated fabrics showed the maximum percentage of reduction with a reduced percentage of 96.87% for *P.putida* followed by 95.12% for *E.coli*. The fabrics without any treatment (control) has negative values for the percentage reduction test because the final number of cells will be much higher than the initial number of cells as it have no bactericidal activity and the results were found to be zero. After ten washing cycles, the herbal nanosilver coated cotton fabrics retain its antimicrobial activity.

DISCUSSION

The herbal nanosilver sample shows peaks at 3313.48, 3193, 2976.90, 2883, 1670, 1452, 1338, 1196.78, and 1112.75cm−1 (Fig. 2). The band at 3,400 cm^{-1} is assigned to the O–H stretching of H-bonded alcohols and phenols. The band at 2,925 cm^{-1} is attributed to O–H stretching of carboxylic acids. The band at 1,616 cm^{-1} corresponds to the N–H bending of primary amines. The band at 3419 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2923 cm^{-1} corresponds to O-H stretch meant for carboxylic acids. The peak value at 1648 cm^{-1} corresponds to N-H bend primary amines. The peak at 1376 cm^{-1} corresponds to C-N stretching of the aromatic amine group and the bands observed in, 1113, 1163, 1059 cm^{-1} corresponds to C-N is stretching carboxylic acids, ethers, alcohols and esters. It will indicate that presence of some proteins, terpinods and alkaloids present on the

surface of the silver nanoparticles, so introduce the new technical term herbal nanosilver.

Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as alkaloids, terpenoids having functional groups of alcohols, ketones, aldehydes and carboxylic acids. From the analysis of FTIR studies confirmed that the carbonyl group from the amino acid residues and proteins has the strong ability to bend metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of silver nanoparticles) to avoid agglomeration and thereby become constant the medium. It indicate that the biomolecules could possibly achieve functions of creation and stabilization of silver nanoparticles and antibacterial and antifungal activities.

The same similar findings by Google et al., (2001) support that proteins can bind to nanoparticles either through the electrostatic attraction of negatively charged carboxylate groups and therefore stabilization of the AgNPs by protein occurs (Saraniya Devi and Valentin, 2012).

Table 2: Testing the efficiency of herbal nano silver loaded cotton fabrics against different bacteria by wash durability test.

S. No.	No. of Washing cycle	Cotton fabrics treated with herbal nanosilver (Percentage of Bacterial reduction)				
		E.coli	***S.aureus***	***P.putida***	***M.leutus***	***P.vulgaris***
1	0	95.12	91.14	96.87	91.34	90.48
2	1	93.85	85.25	81.27	79.52	84.52
3	2	86.25	77.36	67.68	66.51	72.34
4	4	76.96	59.46	47.32	59.16	61.62
5	6	58.47	41.60	28.91	38.75	52.40
6	8	33.87	28.76	15.62	23.12	38.66
7	10	24.11	13.75	7.12	14.16	23.55
8	12	11.85	2.56	0	1.54	12.68
9	14	7.72	0	0	0	5.59
10	16	3.24	0	0	0	0
11	18	0	0	0	0	0

The mechanism of silver antibacterial action is only partially understood. Sondi and Salopek-Sondi (2004) reported that silver nanoparticles interact with building elements of the bacterial membrane, causing structural modifications and degeneration and finally cell death. This is similar to the results of Cho *et al.* (2005) which suggested that the surface cell walls of *S. aureus* and *E.coli* were disrupted by silver nanoparticles. Lee *et al* (2007) investigated the antibacterial effect of nanosized silver colloidal solution against *S. aureus* and *K.pneumoniae* after padding the chemicals on textile fabrics. They reported that the effect was dose dependent and was more pronounced against gram-negative organisms than gram-positive ones. They found that the main mechanism through which silver nanoparticles apparent antibacterial properties was either by anchoring or penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating supposed key peptide substrates on tyrosine residues (Yeo *et al*., 2003). The antibacterial efficacy of the biogenic silver nanoparticles reported in the present study may be ascribed to the mechanism described above but it still remains to clarify the exact effect of the nanoparticles on important c metabolism like DNA, RNA and protein synthesis (Sarkar, 2007).

The result of durability to wash of the treated fabric also showed a long lasting bacteriostatic effect. The *E.coli* was abated on the silver finished textile even after being exposure to 16 consecutive typical careful hand launderings condition. This verifies that nanosized silver particles, as observed in SEM images, were firmly attached onto the fiber surfaces.

CONCLUSION

This study shows that silver nanoparticles synthesized from herbal plant extract *O. sanctum* on cotton fabrics have excellent antibacterial and antifungal activity against *Proteus vulgaris*, *Escherichia coli,* and *Pseudomonas aeruginosa* because the nanosilver surface contains herbal compounds, it was confirmed by FTIR analysis.. The SEM results show the herbal nanosilver have relatively excellent disperses on cotton fabric surfaces.

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