

STUDY ON ANTIMICROBIAL EFFECTIVENESS OF SILVER NANO COATING OVER COTTON FABRIC THROUGH GREEN APPROACH.

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Abstract: In the present investigation the antimicrobial efficiency of cotton fabric treated coated with silver nanoparticles (AgNPs) which was synthesized through biological approach using natural extracts of *Acalypha indica*. The size of silver nanoparticles was found to have ≤ 100 nm. The structure and morphology of silver nanoparticles formed on the cotton fibres were confirmed by electron microscopy. The antibacterial activity of cotton fibres loaded with silver nanoparticles was evaluated against gram-negative *Escherichia coli* (E. coli) bacteria. The results suggest excellent antibacterial activity by the incorporation of 5% leaf extracts on cotton fibres. These fibres have also exhibited superior antibacterial activity indicating their usage in medical and infection prevention applications

INTRODUCTION

Over the last few decades, various research work was happening around the world made to produce antibacterial coated textile materials due to the enormous growth of microbial infections via textile surfaces. (P.N. Danese et al ,2002 & K. Lewiset al 2005).The current research is to develop a non-toxic, cost effective and eco-friendly source of antimicrobial finishing textiles for health care application. Cotton fibres are mostly utilized as raw material towards medical and healthcare products (R. Czajka,2005). However the moisture absorbability of cotton fibres is very high, which makes them more prone to microbial attack under certain conditions of humidity and temperature. Cotton may acts as a nutrient, becoming suitable medium for bacterial and fungal growth (Y. Gao et al,2008). Therefore, cotton fibres are treated with numerous chemicals to get better antimicrobial cotton textiles (Duran N et al 2007 , Son Y.A et al 2006 &Lim S.H 2004). Among the various antimicrobial treatment, nano material based treatment is very effective. Silver nanoparticles (AgNPs) have shown strong inhibitory and antibacterial effects (M. Uchida etal ,1995).As reported by Sondi et al(2004) strong toxicity of silver nanoparticle against wide range of microorganisms is well known. Further he studied the antimicrobial activity of silver nanoparticles against *Escherichia colias* a model of Gram-negative bacteria. Chemical reducing method is one of the important technique followed in synthesise of silver nano particles ,which is normally associated with environmental toxicity(M. Chen et al ,2006).Therefore the development of silver nano particle through natural extract is consider as most important method. As reported by Saifuddin, Biosynthesis of silver nanoparticles using bacteria, fungi, yeast and plants were well documented. Sastry et al.(2003) reported the biosynthesis of nanoparticles through plant leaf extracts and their potential application. They studied bio-reduction of chloraurate ions and silver ions by extracts of geranium and neem leaf. The present study was aimed to synthesis of silver nanoparticles using aqueous leaves extract of *A. indica* and analyse its antibacterial activity against microbes such as such as *Escherichia coli* and *Vibrio cholera*.

PROPOERTIES OF ACALYPHA INDICA

ACALYPHA INDICA L. (family Euphorbiaceae) is a weed widely distributed throughout the plains of India. *Acalypha indica* has been used in ayurvedic system of medicine for various ailments. It has been reported this plants possess hepatoprotective, anti-inflammatory, antitussive, antifungal and used also check wounds healing and antibacterial. It is a common herb growing up to 75 cm tall with ovate leaves. The stem are sparingly to densely hairy. The margins are serrate. They are glabrous and thin. Fruit capsules are small, concealed by the bracts. The seeds are ovoid, smooth, pale brown in colour. Flowers are green, unisexual found in catkin inflorescence. Leaves, stalk, flowers, roots are used as medicine. The Taxonomic classifications are as follows

Taxonomic Classification

Kingdom :Plantae
 Class :Magnoliopsida
 Order :Euphorbiales
 Family :Euphorbiaceae
 Sub family:Acalyphoideae
 Genus :Acalypha
 Species :Acalypha

Raja selvam J et al (2012) revealed that the leaf extract of *Acalypha indica* using different solvent like acetone and aqueous against bacteria strains like *staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella* sp. by agar well diffusion method. The acetone extract of *Acalypha indica* showed the maximum zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis*, minimum inhibition of *Escherichia coli* and *Klebsiella* sp. The aqueous extract of *Acalypha indica* showed maximum inhibition against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. *Klebsiella* sp was resistant to aqueous extract of *Acalypha indica*. Raja, R et al (2013) proved that water extract of *Azadirachta indica* and *Acalypha indica* the plants were more effective than acetone extract particular on *pseudomonas* Sp. Hence both the plants can be vitally used in treating various diseases caused by those pathogens. Ravindra, S et al(2010) proved that 2% leaf extracts of *Acalypha* on cotton fibres exhibits excellent antibacterial activity by the incorporation.

MATERIALS

Plain woven cotton fabric purchased from local fabric suppliers in Dinigul. *Acalypha indica* were collected from campus of Gandhigram Rural university, Dindigul. AgNO₃, acetone were purchased from GVR enterprises Madurai. The bacterial cultures of *E. coli*(MTCC-443) and *V. cholera* (MTCC-3904) were obtained from Department of Biology, Gandhigram University. Dindigul. The other fabric specifications are as follows. Plain weave with well scoured and bleached, EPI- 127, PPI- 95, Warp Count - 66 Ne, Weft Count - 62 Ne.

METHODOLOGY**Collection of Leaves**

Acalypha indica plants are mostly available in roadsides and waste lands. The leaves of *Acalypha indica* are collected from roadsides in and around Trichy. The collected leaves are washed with distilled water for 15 min at the room temperature for the removal of dust and soil particles.

Drying

The collected plants are dried at the room temperature in the open air for one day. It cannot be stored prior to drying to avoid breakdown of important chemical compounds and also it will get contaminated by microorganisms. The dried herbs are kept in a dark room so that the breakdown of important components by sunlight will be prevented.

Grinding

Dry Grinding of the selected herbal portion is powdered in mortar and pestle. After that, the powder is sieved to remove the dirt and unkind particles. The fine powder obtained is used for extraction.



Fig.1. Various forms of *Acalypha indica* plant leaves

Extraction with Solvent Preparation of plant extract:

Aqueous extract of *A. indica* was prepared using freshly collected leaves. They were surface cleaned with running tap water for 15 min at room temperature and dried for 1 day at room temperature. The leaf broth solutions were prepared by taking 3 g, 4g and 5g of washed leaf, followed by distilled water and boiled with 100

ml of distilled water at 60 °C for 5min. This extract was filtered through nylon mesh, followed by Millipore filter (0.45 µ PVDF) and used for further experiments. The extracted leaves solutions were stored at 4 °C and used within a week to produce silver nanoparticles.

Synthesis of Silver Nanoparticles

For synthesis of silver nano particle, the Soxhlet flask containing 100 ml of AgNO₃ (1mM) solution was reacted with selected different leaf broth solution. The resulting solutions were incubated in dark (to minimize the photo activation of silver nitrate), at 37°C under static condition. The observed color change from watery to yellowish brown color solution indicated the formation of *Acalypha indica* -Ag-Np's. The colored Ag-Np's solution was centrifuged at 10,000 rpm for 10 min, the supernatant liquid was decanted. The resulting suspension was redispersed in 10 ml sterile distilled water and centrifugation process was repeated for three times. Thereafter, the purified suspension was used for characterization of Ag-Np's.

Coating of Silver Nanoparticles on Cotton Fabric:

Sliver nano coating over cotton fabric was achieved through leaf broth reduction of Ag⁺ ions. The samples were dipped in 10ml of nano-silver solution and kept on a shaker at 100 rpm for 1 hr at room temperature. After 1 hr, the solution was transferred and the samples were dried at 60°C. The coating of silver nanoparticle on cotton fabric is also similar to situ synthesis of silver nanoparticle on cotton that is the hydroxyl groups of leaves extracts reduce the silver nitrate into silver nanoparticles on cotton and then simultaneously coated over the fibre surface. During this reaction the abundant hydroxyl groups of cotton fibre facilitate the surface adsorption of silver nanoparticle on cotton.

Characterization of silver nanoparticle

Synthesized silver nanoparticles was confirmed by UV spectra analysis. The reaction mixture samples were studied at regular intervals and the absorption maxima was scanned by UV-vis spectra, at the wavelength of 200–600nm in Beckman-DU 20 spectrophotometer. The formation of silver nanoparticles were confirmed by UV-Spectroscopy. As reported by N Duran et al(2007), the silver nanoparticles exhibit ruby red color in water, having an intense absorbance band around 400–450nm arising due to surface plasmon excitation vibrations in the metal nanoparticles. The figure 2 illustrates the UV spectra produced during the conversion of silver nanoparticles using 3g, 4g and 5g of *A.Indica* leaves extracts. The results showed that the maximum conversion of silver nanoparticles has taken place. The intense peaks in the UV Spectra were observed at 422.432 and 438 nm in the case of 3g, 4g, 5g of leaves extract. More over the results further confirms that 5% concentration of *A.indica* extract converted the maximum silver ions into silver nanoparticles

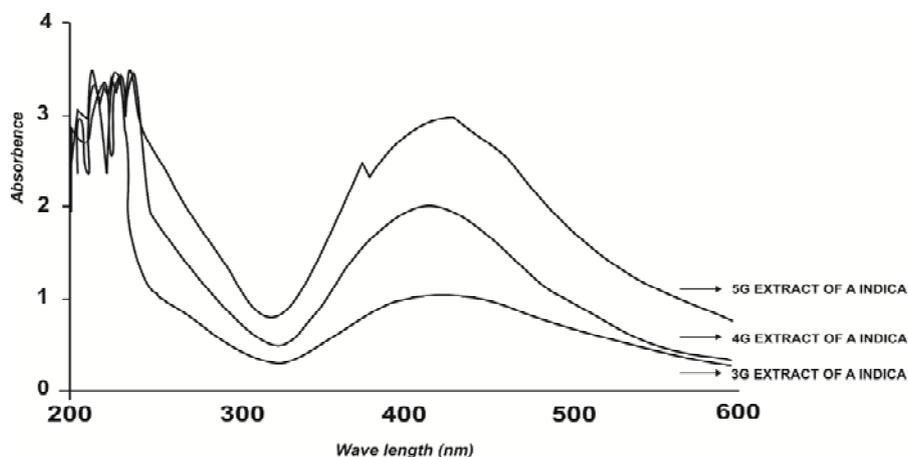


Fig.2. UV- spectra of nanoparticles formed using 3, 4, and 5% *A. Indica* leaves extract solutions

SEM ANALYSIS COATED TEXTILES

The morphological feature of synthesized silver nanoparticles from *acalypha indica* plant extract has been coated over the cotton textile was studied through Scanning Electron Microscope (JSM-6480 LV). The SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV. SEM analysis of the synthesized silver nanoparticles was clearly distinguishable owing to their size difference. The size of the formed nanoparticle would be in the range of 100-150 nm. The schematic representation of the formation of silver nanoparticles on cotton fabric is presented. The EDS spectrum (Fig4) recorded from silver nanoparticles showed strong signal of silver nanoparticle.

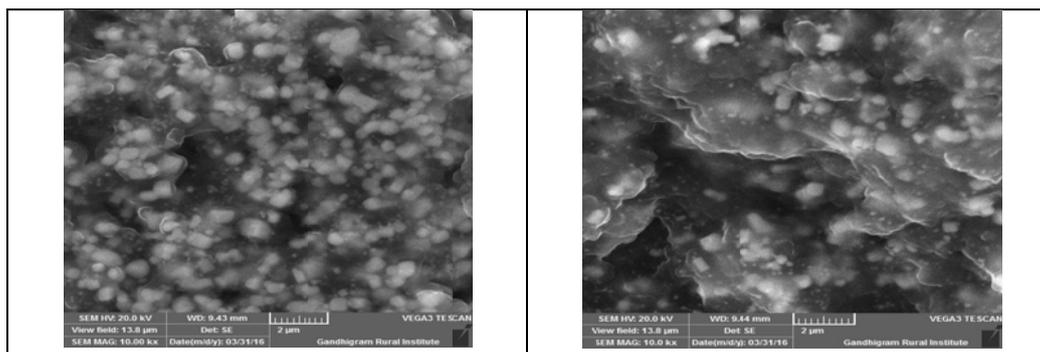


Fig.3.SEM images of silver nanoparticles coated over the cotton textile fabric

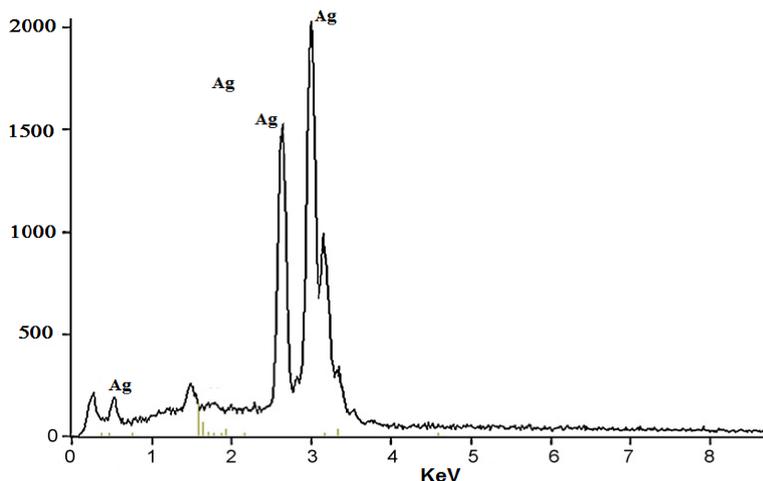


Fig.4.EDS analysis of silver nanoparticles.

RELEASE OF Ag NANO PARTICLE

It is one of the important aspects to measure the antimicrobial activity of over a period of time, which has been studied through release test. To exert the efficient antimicrobial activity over a period of time was achieved through the coated silver nanoparticle is very important. In general silver nanoparticles are non-reactive but in aqueous media it releases silver ions which are responsible for the antimicrobial activity of that structure. To do this experiment the coated textile structure was immersed in to the saline solution at 37° C. Regular time intervals the % of Ag Np release were interpreted through UV-Vis spectra. The fig. 5 shows the in vitro release pattern of silver nanoparticle by varying the percentage of A.Indica extract. In comparison with the all the combination 5 g extract based coating yields faster release compare with other NP's loaded cotton fabrics. The release study was observed over the period of 200 hrs. The cumulative average Ag NP's release of all the combination produces 42.13% during the entire release study period. The prolonged release of silver nanoparticle helps to produce the cure the wound for long period of time.

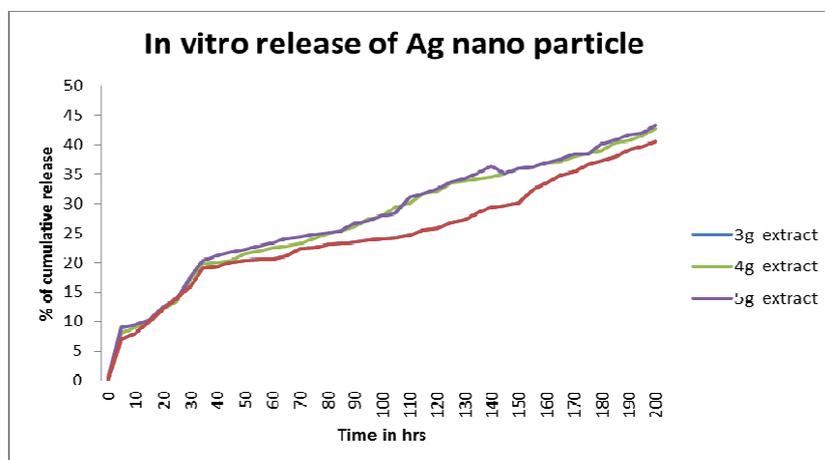


Fig.5. In vitro release of Ag nanoparticle coated on cotton fabric

ANTI-MICROBIAL ACTIVITY

The antibacterial activity of *A. indica* -Ag-Np's was evaluated against both Gram positive and Gram negative (bacillus cereus, klebsiella pneumonia, candida albicans, pseudomonas aeruginosa, candida glabrata) pathogenic microorganisms by disc diffusion method. The antimicrobial activity of the coated silver nanoparticles on cotton fabrics was measured as zone of inhibition in mm (diameter size) as given in fig.7.

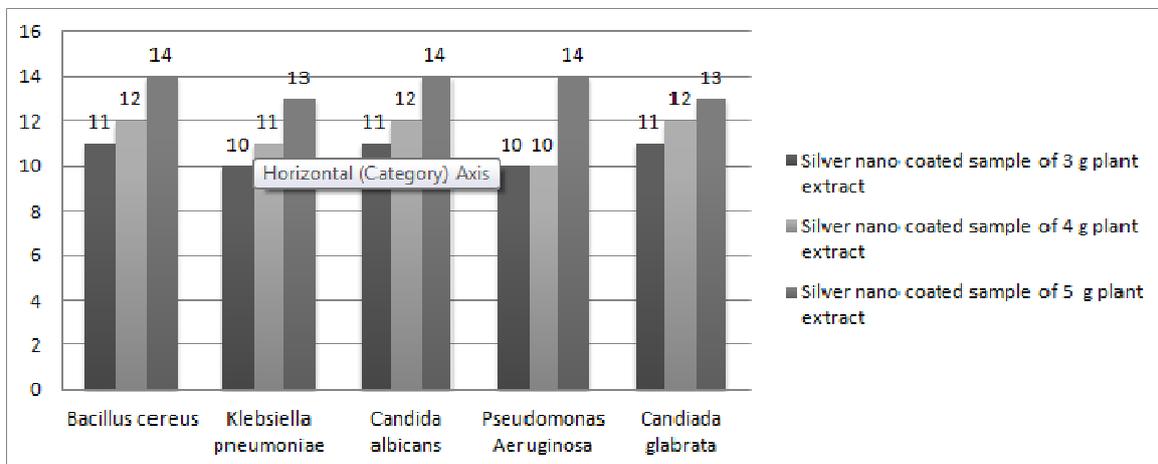


Fig.6. Zone of inhibition (mm) Silver nano coated cotton sample against various Bacterial species

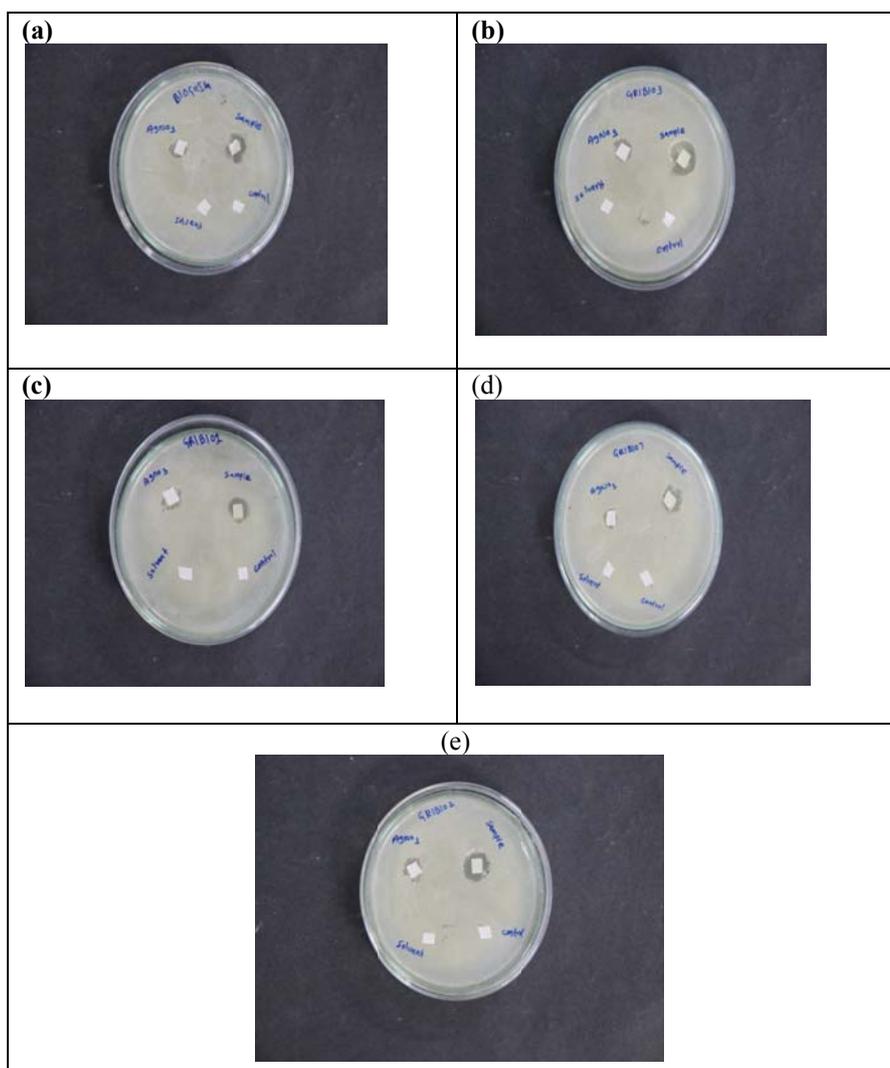


Fig.7. Antimicrobial tested Sliver nano-coated cotton fabric samples using 5 g plant extract against various biological species (a) Pseudomonas aeruginosa (b) Candida albicans (c) Bacillus cereus (d) Cadida glabrata (e) Klebsiella pneumonia

Sliver nanoparticles displayed almost similar range of antimicrobial activity against studied pathogens, which was understood through diameter of inhibition zone. That is zone of inhabitation value in the range of 12-14 mm. Several studies were concluded the biocidal properties of silver nanoparticles against microorganisms. It is believed that the silver nanoparticles attach the negatively charged cell surface, then change its physical and chemical properties of the cell membranes and the cell wall and disturb the permeability, osmoregulation, electron transport and respiration (Marambio-Jones, C., & Hoek, 2010). Second the silver nanoparticle produces further damage by permeating into the cell, interact with the DNA (AshaRani, P. V et al, 2009). Third the silver nanoparticle releases the silver ions producing higher biocidal effect on the microorganisms. In the selected cases higher bactericidal activity achieved through 5g A.Indica extract as compare with the other two cases. This higher activity is due to the higher amount of Ag nanoparticles on the surface of the cotton fabric. Still further improvement has not been achieved by increasing more than 5g A.Indica extract, which was attributed that optimum loading of Ag nanoparticles were achieved through 5g A.Indica extract itself.

CONCLUSION

From the present research it is proved that biosynthesized silver nanoparticles using A.Indica leaf extract was possible and which can be coated over cotton fabric through

in situ chemical reaction. SEM image, UV spectra proved that the formation of silver nanoparticles. The biological approach is a cost effective method as compare with the chemical synthesis. Further it is proved that the silver nanoparticles coated cotton fabric exhibit effective antimicrobial effect against microorganism. Finally the 5g A.Indica leaf extract produces highest antimicrobial effect and release properties as compared with 3g and 4g leaf extracts. Due to the highest control release properties of this coating utilized for wound healing dressing.

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