Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 4, 2011

Research Article

SILVER NANOPARTICLE SYNTHESIS FROM *LECANICILLIUM LECANII* AND EVALUTIONARY TREATMENT ON COTTON FABRICS BY MEASURING THEIR IMPROVED ANTIBACTERIAL ACTIVITY WITH ANTIBIOTICS AGAINST STAPHYLOCOCCUS *AUREUS* (ATCC 29213) AND E. COLI (ATCC 25922) STRAINS

S.KARTHICK RAJA NAMASIVAYAM AND AVIMANYU

Department of Biotechnology, Sathyabama University, Chennai 600119, Tamil Nadu, India. Email: biologiask@gmail.com

Received: 24 June 2011, Revised and Accepted: 3 Aug 2011

ABSTRACT

In the present work, silver nanoparticles synthesized by *Lecanicillium lecanii*, were coated on the bleached cotton fabrics using acrylic binder adopting pad dry method and anti bacterial properties of coated fabrics were studied against pathogenic bacteria *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) Further treatment involves measuring of improved antibacterial activity of.nanoparticles coated cotton fabrics with tetracycline, ofloxacin and neofloxin against *E.coli* , cloaxicillin and ofloxacin against *S.aureus* at 20,40 and 60 µg concentration. Silver nanoparticles synthesized extracellularly by *L.lecanii* and the synthesized particles were characterized by **.** UV-Vis spectra and scanning electron microscopy which reveals strong silver plasmon absorption maxima at 410 nm and spherical nanoparticles with the size of 45- 100nm respectively. The surface topography of silver nanoparticles coated cotton fabrics was observed under scanning electron microscopy reveals complete dispersion of silver nanoparticles on the fiber surface and the size of the embedded particles was 45-100nm. Nanoparticle coated cotton fabrics at all the tested concentration inhibited both the tested pathogenic bacteria. Furthermore silver nanoparticles with the tested antibiotics coated cotton fabrics showed increased spectrum of anti bacterial activity. Antibacterial activities of all antibiotics have been increased against both the test strain with minimum 5 mm to maximum 10mm. The highest increase in inhibitory zone for *E.coli* and *Staph.aureus* was observed against neoflaxin and ofloxacin. Synergic effect of silver nanoparticles on the antibacterial activity of remaining antibiotics against the both tested strains was observed These results signify that the silver nanoparticle potentate the antimicrobial action of the antibiotics and the possible utilization of nanocompound in combination effect against pathogenic bacteria causing sever wound infections.This study will lead to new generation of development of dressing incorporating antimicrobial agents to prevent pathogens infection

Keywords: Silver nanoparticles, *Lecanicillium lecanii,* Fabric, Antimicrobial Activity

INTRODUCTION

An important area of research in nanotechnology is the synthesis of nanoparticles of different chemical compositions, sizes, shapes and controlled disparities. Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. There is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. It is well known that many microorganisms like algae, bacteria and fungi produce nanoparticles either intracellularly [1] or extracellularly [2]. Since noble metal nanoparticles such as silver, gold nanoparticles are widely applied to human contacting areas. Silver nanoparticles have been reported to have antimicrobial activity against a wide range of microorganism.Taken together, this compound as a highly safe compound may be considered for combination therapy against pathogenic microorganism due to its potential synergistic effect with important antibiotics. These nanoparticles exhibit tolerable monodispersity and in the case of particles synthesized extracellularly, exhibit excellent long term stability. Silver nanoparticles have been reported to have antimicrobial activity against a wide range of microorganisms [3]. Taken together, this compound as a highly safe compound may be considered for combination therapy against pathogenic microorganism due to its potential synergistic effect with important antibiotics. With the advent of science and technology, a new area has developed in the realm of textile finishing. Nanocoating the surface of textiles, clothing, and textiles for footwear is one approach to the production of highly active surfaces to have UV-blocking, antimicrobial and self-cleaning properties. The self-cleaning property can be impacted by nano-TiO2/nano-ZnO coating [4] while nano silver imparts antimicrobial activity [5].These particles can be incorporated in several kind of materials such as cloths. These cloths with metallic nanoparticles are sterile and can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus*. Enhancement of textile materials by nanotechnology is expected to become a trillion dollar industry in the next decade with tremendous technological, economic and ecologic benefits.Antibacterial agents were used on textiles thousands of years ago, when ancient Egyptians used spices and herbs as preservatives in mummy wraps [6] In order to explore the potential of nanoparticles coated fabrics to protect against infectious agents, a study was undertaken to evaluate (1) anti bacterial activity against pathogenic bacteria *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) (2) synergistic effect of antibiotics tetracycline, ofloxacin and neofloxin against *E.coli* , cloaxicillin and ofloxacin against *S.aureus* .

MATERIALS AND METHODS

Fungal strain

Lecanicillium lecanii (MTCC 2067), was obtained from Microbial type culture collection (IMTECH) Chandigarh, India and the strain was maintained in Sabouraud Maltose Yeast Extract Agar (SMYA) slant.

Synthesis and characterization of silver nanoparticle

The fungal inoculum was prepared in 250 ml of Sabouraud Maltose Yeast Extract Broth (malt extract- 0.3g, yeast extract-0.3g, peptone-0.5g, glucose- 1g, agar- 2g, distilled water- 100ml, pH- 7) at 28^{0}C for 6 days. After the incubation period, biomass was filtered and resuspended in sterile water and 10 gm of washed fungal biomass was taken in a conical flask containing 100 ml sterile Millipore water at 28^{0}C under shaking condition for 3 days. The aqueous solution component was separated by filtration. $AgNO_3$ (10^{-3}M) was added to the filtrate and kept at 28^{0}C under shaking condition, periodically aliquot of the reaction solution was removed and the absorption was measured in UV-Vis spectrophotometer at 410-420 and scanning electron microscopy.

Coating of silver nanoparticles and antibiotics on cotton fabrics and evaluation of antibacterial activity

The sterile (100% cotton woven fabric (plainweave, 75×30 g/m2; ends, 75/inch; picks, 60/inch) was used for the application purpose. Silver nanoparticles were applied on cotton using pad-dry-cure method [6].The cotton fabrics cut to the size of 10 ´ 10 cm was immersed in the solution containing silver nanoparticles and the respective antibiotics at 20,40,60 µg and acrylic binder (1%) for 5 minutes then it was passed through a padding mangle, which was running at a speed of 15 m/min with a pressure of15 kgf/cm2 to remove excess solution. A 100% wet pick-up was maintained for all of the treatments. After padding, the fabric was air-dried and then cured for 3 min at140°C. The fabric was then immersed for 5 min in 2 g/l of sodium lauryl sulfate to remove unbound nanoparticles and antibiotics .Then the fabric was rinsed at least 10 times to completely take out all the soap solution. The fabric thus washed was air-dried and dried pieces were used for antibacterial activity.

Characterization of nanocoated fabrics by SEM

The surface topography of silver nanoparticles coated cotton fabrics was observed under scanning electron microscopy.

Antibacterial activity of synthesized nanoparticles

The antibacterial activity of nanoparticle was tested against pathogenic bacteria *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), obtained from American Type Culture Collection. The strains were maintained on brain heart infusion agar slants. A single colony of the test strains were grown overnight in Mueller Hinton broth on a rotary shaker (200 rpm) at 35°C. The inoculums were prepared by diluting the overnight cultures with 0.9%NaCl to a 0.5 McFarland standard. A lawn of the test organism was made on the agar. The dried cotton fabrics coated with nanoparticles, placed on the swabbed plates. The seeded plates were incubated at 37^0C for 24 hours and the plates were observed for zone of inhibition. After the incubation period the diameter of the zone was recorded.

Evaluation of improved activity of antibiotics coated with silver nanoparticles

Antibiotics

The improved activity of silver nanoparticles with antibiotics which were obtained from Hi media, Mumbai,India was carried out with coating of silver nanoparticles at different concentration (20,40 and 60 µg which were originally prepared from stock solution) with tetracycline, ofloxacin, neofloxin against *E.coli,* cloaxicillin and ofloxacin against *Staphylococcus aureus* by pad dry care method and evaluated against pathogenic bacteria as described earlier.

RESULT

Synthesis and characterization of silver nanoparticles

The synthesis of nanoparticles was confirmed initially by colour change of reaction mixture from pale yellow to brown which was observed after the seventh day of incubation (Fig 1). The brownish colour formation is clear indication of silver nanoparticle in the reaction mixture. Moreover the reaction mixture was remaining as brown colour for months.

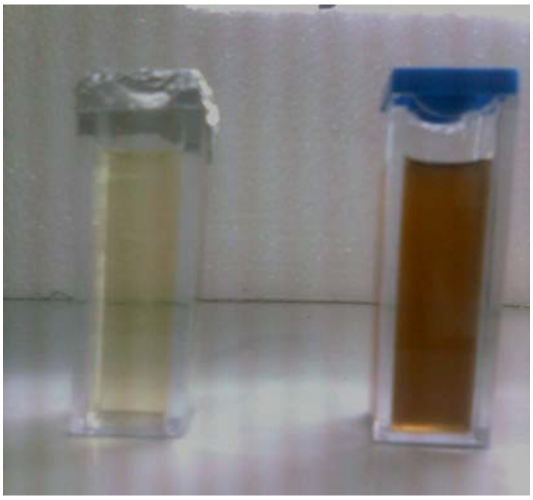


Fig. 1: Reaction mixture with silver nanoparticles synthesized by *L.lecanii*

No aggregation and turbidity was observed in the entire synthesized nanoparticle which reveals the stability and the synthesized nanoparticles were further characterized by UV-Vis spectrophotometer and scanning electron microscopy (SEM). A strong silver Plasmon absorption maxima was recorded at 410-420 nm in UV-Vis spectrophotometer (Fig.2).

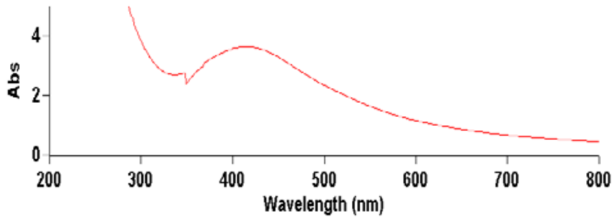


Fig. 2: UV vis absorption spectra of silver nanoparticles

Further characterization was carried out using SEM which reveals spherical silver nanoparticle with the size range of 60-100nm (Fig.3).

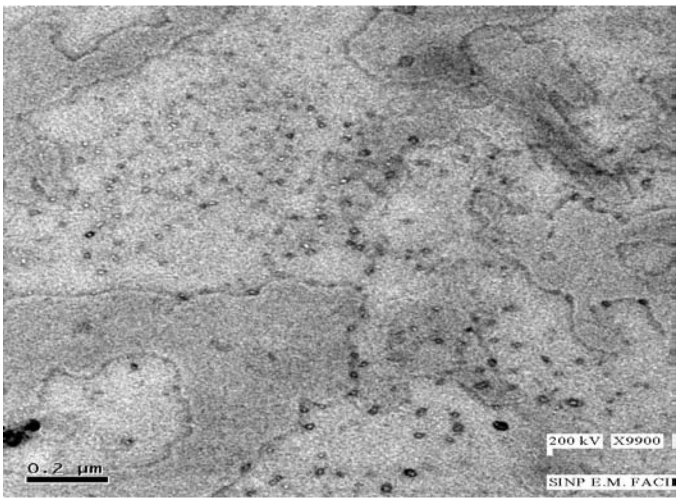


Fig. 3: Scanning electron micrograph of silver nanoparticles

Topographical analysis of nano silver cotton fabric

The surface of treated cotton fabric was analysed by scanning electron microscope to observe the size and shape of the nanoparticles. The nanoscaled silver particles were observed on surface of the cotton fabric.

The nanoparticles were well dispersed on the fibre surface. Scanning electron microscope analysis was done to measure the size of nanoparticles. In this analysis 60-100 nm sized silver nanoparticles were obtained. The particle size plays a primary role in determining their adhesion to the fibre. It is reasonable to expect that the largest particles agglomerates will be easily remove from the fibre surface, while the small particles will penetrate deeper and adhere strongly into fabric matrix. The SEM analysis of the treated fabrics showed nano particles embedded on to the fabrics (fig.4).

Antibacterial activity of silver nanoparticles and antibiotics coated cotton fabrics

Antibacterial activity of cotton fabric coated silver nanoparticles showed distinct bacteriacidal effect against both the tested strains with all the tested concentration (Table 1)

12, 13 and 14 mm zone of inhibition was recorded in *E.coli* (Figure 5a) at the concentration of 20, 40, 60µg. In *Staph.aureus* 11.5, 13.0 and 14.0 mm of zone of inhibition recorded at respective concentrations (Figure 5b).

Antibiotics coated cotton fabrics inhibited both the tested pathogens at all the tested concentration (Table 2).

offlaxacin at all the concentration revealed maximum zone of inhibition against *E.coli* (Figure 6) whereas cloaxacillin against *Staph.aureus* (Figure 7)

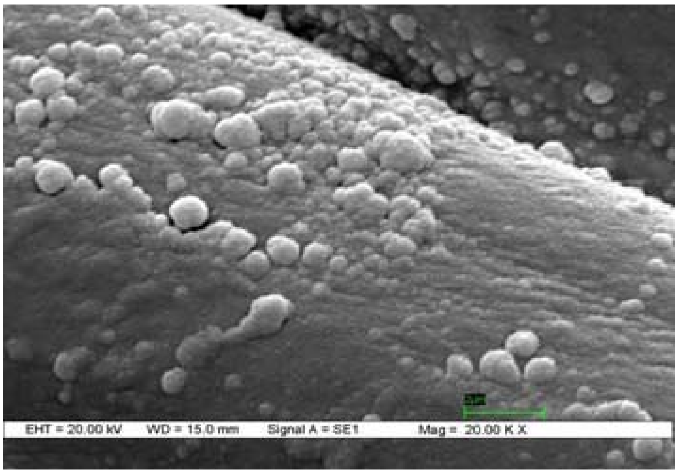
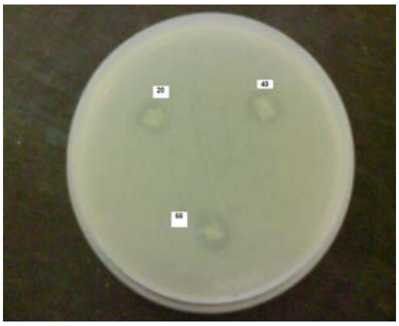


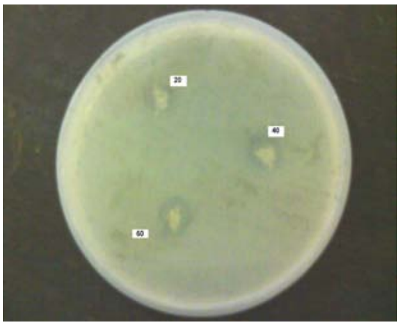
Fig. 4: Scanning electron micrograph of cotton fabric coated with silver nanoparticles

Table 1: Zone of inhibition (mm) of silver nanoparticles of different concentration (µg) against pathogenic bacteria

Sl. No	Concentration(µg/ml)	Zone of inhibition(mm)	
		E.coli	*S.aureus*
1	20	12	11.5
2	40	13	13
3	60	14	14



(a)

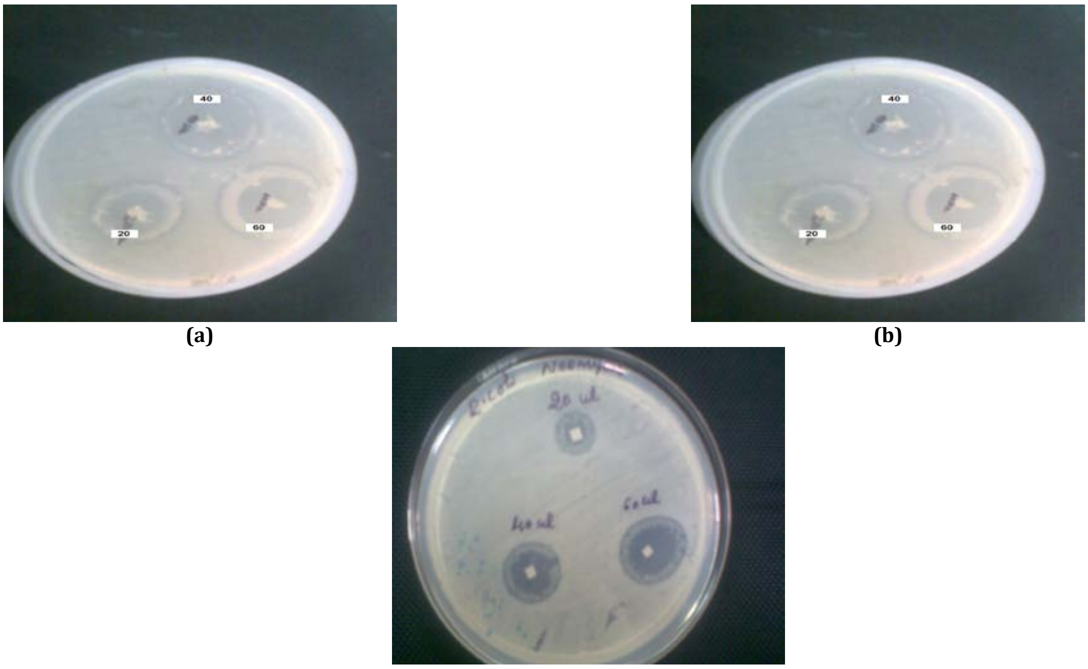


(b)

Fig. 5: Zone of inhibition with silver nanoparticles coated cotton fabric against (a) *E.coli, (b) Staphylococcus aureus*

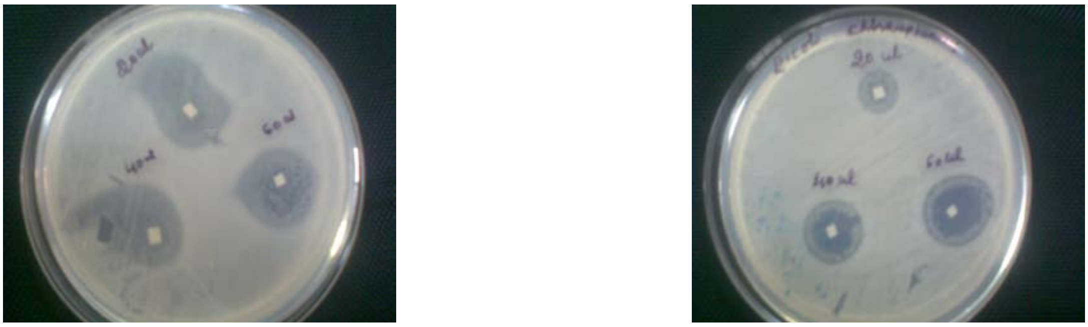
Table 2: Zone of inhibition (mm) with antibiotics coated cotton fabrics against tested organism

S. No	Organisms tested	Antibiotics Used	Zone of Inhibition(mm)		
			20µg	40 µg	60 µg
1	*E.coli*	Tetracycline	11	13	14
2		Ofloxacin	12	14	16
3		Neoflaxin	12	14	15
4	*S.aureus*	Cloaxicillin	13	15	16
5		Ofloxacin	12	14	15



(a) **(b)** **(c)**

Fig. 6: Zone of inhibition with (a) tetracycline (b) offloxacin (c) neoflaxin against *E.coli*



(a) **(b)**

Fig. 7: Zone of inhibition with (a) cloaxicillin (b) offloxacin against *Staph.aureus*

Improved activity of antibiotics with silver nanoparticles coated cotton fabrics

In both the tested strains, distinct variation in antibacterial activity of antibiotics with silver nanoparticles was observed against both the tested strains (Table 3).

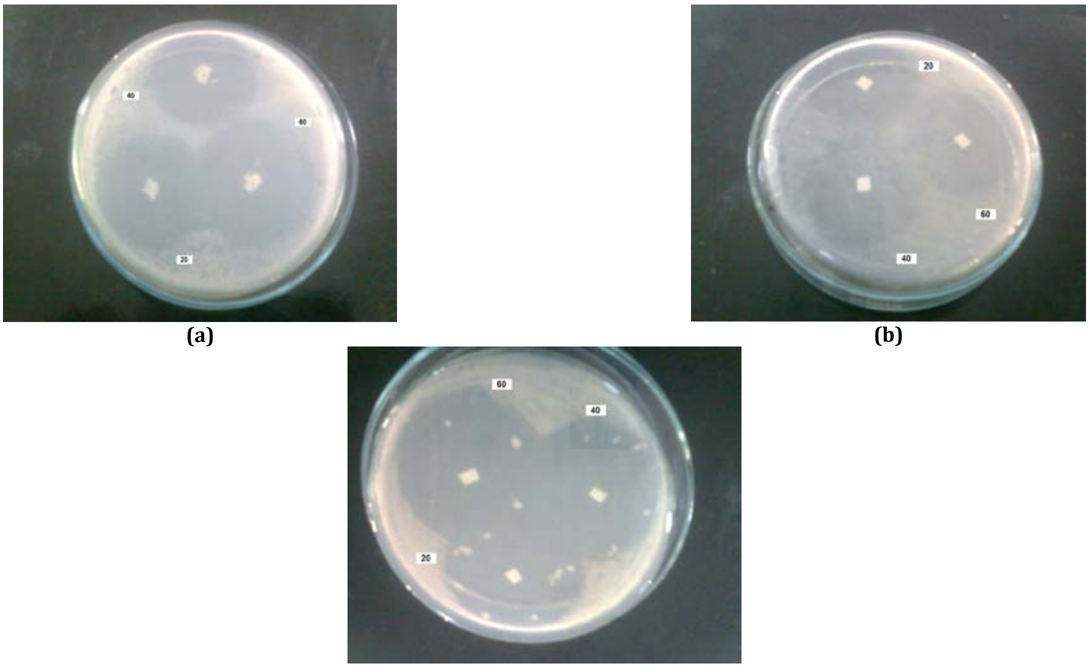
In *E.coli*, 11.0 mm, 14.0 and 16.0 mm of zone of inhibition was observed at 20, 40 and 60µg of concentrations of ofloxacin without nanoparticles (Fig.7)

But the zone of inhibition was increased as 27mm, 31mm and 34nm in ofloxacin with silver nanoparticles (Fig.b). Neoflaxin without silver nanoparticles recorded 12.0 mm, 14.0 mm, and 15mm of zone of inhibition and 31mm, 34.0 mm and 37.0 nm was in neoflaxin loaded silver nanoparticles (Fig.7c). 12.0, 14.0 and 14.0 mm of zone of inhibition in tetracycline (Fig.6a) was increased to 30mm, 33mm and 35mm in tetracycline with silver nanoparticles (Fig.7a).

In the case of *S.aureus* , increased antibacterial spectrum was observed in antibiotics loaded silver nanoparticles applied cotton fabrics.Antibiotics without nanoparticles showed less zone of inhibition at all the concentration tested. (Table 2) 13.0,15.0 and 16.0mm, and 12.0mm, 14.0 and 16.0 mm of zone of inhibition was recorded in cloaxicillin and ofloxacin without silver nanoparticles respectively (Fig.8). cloaxicillin with silver nanoparticles recorded 28.0,33.0,36.0 mm of inhibition. 30.0, 33.0 and 36.0mm, of zone of inhibition was recorded in ofloxacin with silver nanoparticles(Fig.8).

Table 3: Zone of inhibition (mm) with nanoparticles with antibiotics scoated cotton fabrics against tested organism

S.No	Organisms tested	Antibiotics +Ag Nanoparticles	Zone of Inhibition(mm)		
			20µg	40 µg	60 µg
1	*E.coli*	Tetracycline	30	33	35
2		Ofloxacin	27	31	34
3		Neoflaxin	31	34	37
4	*S.aureus*	Cloaxicillin	28	33	36
5		Ofloxacin	30	33	36



(a) **(b)** **(c)**

Fig. 7: Zone of inhibition with silver nanoparticles with (a) tetracycline (b) ofloxacin (c) neoflaxin against *E.coli*

(a) **(b)**

Fig. 9: Zone of inhibition with silver nanoparticles with (a) cloaxicillin (b) ofloxacin against *Staph.aureus*

DISCUSSION

Due to the antibiotic resistance developed by the bacteria it is very hard to manage the different types of antibacterial drugs and there is no suitable long lasting vaccine available. So, there is great need of agents to be able to kill bacteria and other microorganisms. Because of the unavailability of drug the world authorities considered the different types of antibiotics and disinfectants [7].The emergence of bacterial resistance to antibiotics and its dissemination, however, are major health problems, leading to treatment drawbacks for a large number of drugs and most of the drugs are fail to treat the diseases because of their poor pharmaceutics characteristics. Hence, there has been increasing interest in the use of novel effective drugs and their best compatibility with other chemptherapeutic agents as combination therapy against the disease [8].Special drug carrier systems such as nanoparticles hold the promise of to fulfil such a unique pharmacokinetics characteriatics which bring about successful therapy .Nanoparticles are stable, solid colloidal particles consisting of macromolecular material and ranging in size from 10 to 1,000 nm. Drugs can be adsorbed on the particle surface or can be entrapped or dissolved in the particle matrix [9] various techniques for the preparation of nanoparticles have been employed for a large number of anti microbial drugs. Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol[2]

There is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals Silver nanoparticles have been reported to have antimicrobial activity against a wide range of microorganism [3] .Taken together, this compound as a highly safe compound may be considered for combination therapy against pathogenic microorganism due to its potential synergistic effect with important antibiotics. In recent years noble metal nanoparticles have been the subjects of focused researches due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. Silver containing materials can be employed as antibacterial agent.. A new generation of dressing incorporating antimicrobial agents consist of silver nanoparticles with antibiotics showing synergistic effect will reduce or prevent infections caused by pyogenic infection causing organism.In this study,silver nnaoparticles synthesized extracellularly coated on cotton fabric by pad care dry method was evaluated against *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) with antibiotics tetracycline, ofloxacin and neoflaxin (*E.coli*) cloaxicillin and ofloxacin (*S.aureus*) reveals the distinct dispersion and binding of nano silver with the diameter of 45-100nm on cotton fiber and distinct anti bacterial activity against both the tested strain. Improved anti bacterial activity was observed in all the tested antibiotics with silver nanoparticles coated cotton fabric.Similar finding was reported by Thirumurugan *et al* [9] They observed increased spectrum of antibacterial activity of cloaxicillin and tetracycline with silver nanoparticles against methicillin resistance *Staphylococcus aureus*. Improved antibacterial activity of quinolone capped colloidal gold nanoparticles against *Staphylococcus aureus, Micrococcus luteus, Escherichia coli and Pseudomonas aeruginosa* was reported by Grace *et al* [3] Venubabu Thati *et al* [10] also observed distinct antibacterial activity of antibiotics with zinc oxide nanoparticles against *S.aureus*. Antibacterial activity of cotton fabrics impregnated with silver nanoparticles against *Staph.aureus* synthesized from *Fusarium oxysporum* was reported by Marcato *et al* [7]

The present study demonstrated that silver nanoparticles synthesized extracellularly by *Lecanicillium lecanii* incorporated in several kinds of materials such as cloths. This cloth with nano silver can be used in hospitals to prevent or to minimize bacterial infections and will lead to new generation of development of dressing incorporating antimicrobial agents to prevent pathogens infection.

ACKNOWELEDGEMENT

We acknowledge Indian institute of Technology (IIT), Chennai,Tamil Nadu, India for SEM analysis of silver nanoparticless.

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