

## ANTIMICROBIAL PROPERTIES OF LANTHANUM ALUMINATE NANOPARTICLES

Gayathri S<sup>1</sup>, R. Ranjithkumar<sup>2</sup>, A.S. Balaganesh<sup>1</sup> and B. Chandar Shekar<sup>1\*</sup>

<sup>1</sup>Department of Physics, Kongunadu Arts and Science College, Coimbatore-29.

<sup>2</sup>The NEAR Foundation, The Nilgiris, Tamilnadu, India.

\*Email: chandar.bellan@gmail.com

### ABSTRACT

The sol-gel route synthesized LA-NPs were tested for antimicrobial properties against different human pathogenic bacteria and fungi. The test organisms used were clinical isolates viz., *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella nemoniae* and the human fungal pathogens like *Candida albicans* and *Trichoderma viride*. The LA- NPs achieved maximum activity against *S. aureus* compared with other three tested organisms such as *S. pyogenes*, *E. coli* and *K. pneumonia*. It also showed very good antimicrobial properties against studied fungi. At the concentration 1 mg/ml LA-NPs impregnated filter paper disk achieved maximum activity against human pathogen.

**Keywords:** Antimicrobial activity, Lanthanum aluminate nanoparticles, human pathogens.

### 1. INTRODUCTION

The application of nanomaterials in biotechnology merges the fields of material science and biology. Nanoparticles provide a mostly useful platform, demonstrating distinctive properties with potentially wide-ranging applications in therapeutic field (Gao *et al.*, 2004). The advancements in the area of nanoparticles technology and nanotechnology have offered an understanding and controlling of the materials at atomic and molecular levels. It has also assisted in fabricating advanced materials with added optical, electrical, magnetic and biological properties for pharmaceutical and biomedical applications (Iconaru *et al.*, 2012). Nanovectors in the field of delivery are promising novel tools for controlled release of drug (Maya *et al.*, 2015). Bio macromolecule external recognition by nanomaterials as artificial receptors provides a potential tool for controlling cellular and extracellular processes for numerous biological applications such as enzymatic inhibition, transcription regulation delivery and sensing. The biological application of nanoparticles depending on the core size of material providing a suitable platform for the interaction of nanomaterial with biomolecules (Hostetler *et al.*, 1998). Nanomaterials have already been used for a wide range of applications both *in-vitro* and *in-vivo*. The surface and core properties of nanomaterials can be engineered for individual and multimodal applications, including biomolecular recognition, therapeutic delivery such as antimicrobial, anticancer, biosensing and bio imaging (Mrinmoy *et al.*, 2008).

In this world of emerging nanotechnology, one of the primary concerns is the potential environment impact of nanoparticles. An efficient way to estimate nanotoxicity is to monitor the response of bacteria exposed to these particles. Resistance of bacteria to bactericides and antibiotic has increased in recent years due to the development of resistant strains. Some antimicrobial agents are extremely irritant and toxic and there is much interest in finding ways to formulate new type of safe and cost-effective biocidal materials (Brayner, 2008). Earlier studies have been shown that antimicrobial formulations in the form of nanoparticles could be used as effective bactericidal materials. Recently, it has been reported that highly reactive metal oxide nanoparticles exhibit excellent biocidal active against Gram positive and Gram negative bacteria (Kim *et al.*, 2007; Savithramma *et al.*, 2011; Kagan *et al.*, 2002).

Bacteria are generally characterized by a cell membrane, cell wall, and cytoplasm. The cell wall lies outside the cell membrane and is composed mostly of a homogeneous peptidoglycan layer. The cell wall maintains the osmotic pressure of the cytoplasm as well the characteristic cell shape. Gram positive bacteria have one cytoplasmic membrane with multilayer of peptidoglycan polymer and a thicker cell wall (20-80 nm). Whereas gram-negative bacteria wall is composed of two cell membranes, an outer membrane and a plasma membrane with a thin layer of peptidoglycan with a thickness of 7-8 nm. Nanoparticles size within such ranges can readily pass through the peptidoglycan and hence are highly susceptible to damage (Fu *et al.*, 2005;

Amna *et al.*, 2015). Hence, the preparation, characterization and surface modification of nanosized particles open the possibility of formulation of a new generation of bactericidal materials (Duncan, 2011). The nanoparticles present a highly attractive platform for a diverse array of biological applications. Hence the present study focuses the antimicrobial properties of sol gel route synthesized Lanthanum Aluminate Nanoparticles (LA-NPs) against different human pathogenic bacteria and fungi.

## 2. MATERIALS AND METHODS

### 2.1. Lanthanum Aluminate Nanoparticles (LA-NPs)

Our research group already reported synthesis of LA-NPs by sol-gel method and obtained nanoparticles were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM) and Energy Dispersive Spectrum (EDS) (Gayathri and Chandar Shekar, 2015). The synthesized LA-NPs were tested for antimicrobial properties against different human pathogenic bacteria and fungi.

### 2.2. Test microorganisms

The test organisms used were clinical isolates *viz.*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. The human fungal pathogens like *Candida albicans* and *Trichoderma viride*, which were obtained from Department of Microbiology, Raja Muthaiyah medical college, Annamalai University. The bacterial and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively. The bacterial cultures were maintained on nutrient broth (Table I) at 37°C and fungus was maintained on Potato dextrose agar (Table. II) at 28°C.

**Table 1. Composition of Nutrient Broth (NA) medium**

Peptone	5.0 g
Beef extract	3.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	7.0

**Table 2. Composition of Potato Dextrose Agar (PDA) medium**

Potato	200.0 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	6.2

### 2.3. Preparation of inoculum

The gram positive bacteria *Streptococcus pyogenes* (*S. pyogenes*), *Staphylococcus aureus* (*S. aureus*) and gram negative bacteria *Escherichia Coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) were pre-cultured in Nutrient Broth (NB) over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically ( $A_{610}$  nm). The fungal inoculums *Candida albicans* (*C. albicans*), *Trichoderma viride* (*T. viride*), were prepared from 5 to 10 day old culture grown on Potato Dextrose Agar (PDA) medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer ( $A_{595nm}$ ) to obtain a final concentration of approximately  $10^5$  spores/ml.

### 2.4. Anti-bacterial activity

The antibacterial activities of sol-gel route synthesized nanoparticles were tested against both gram positive and gram negative human pathogens by the standard disk diffusion method. In brief, different concentration nanoparticles were prepared by reconstituting with distilled water. The test human pathogens were seeded into respective medium by spread plate method 10  $\mu$ L (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper disks (4 mm in diameter) were impregnated with different concentration of LA-NPs (0.5 mg/ml and 1mg/ml). Followed by this step, the nanoparticles impregnated filter papers were placed on test organism-seeded plates. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mille meter (mm).Chloramphenicol (10  $\mu$ g) used as standard for antibacterial test.

### 2.5. Anti-fungal activity

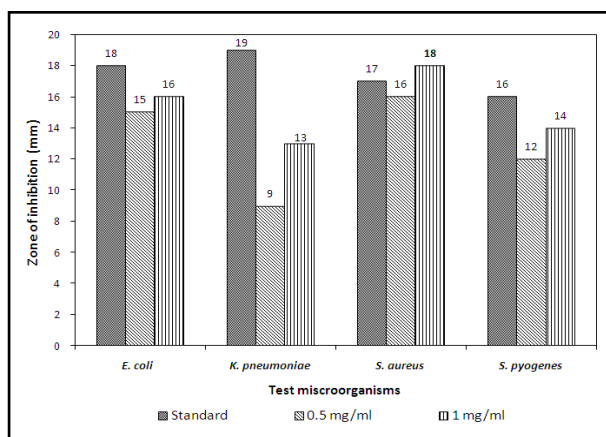
The antifungal activities of sol-gel route synthesized nanoparticles were tested against two pathogens by the standard disk diffusion method. In brief, the potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (4 mm in diameter) impregnated with different concentrations (0.5 mg/ml and 0.1 mg/ml) of LA-NPs. Followed by this step, different concentration nanoparticles impregnated filter paper disk were placed on test organism-seeded plates. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were

measured in mm. Chloramphenicol (10 µg) used as positive control.

### 3. RESULTS AND DISCUSSION

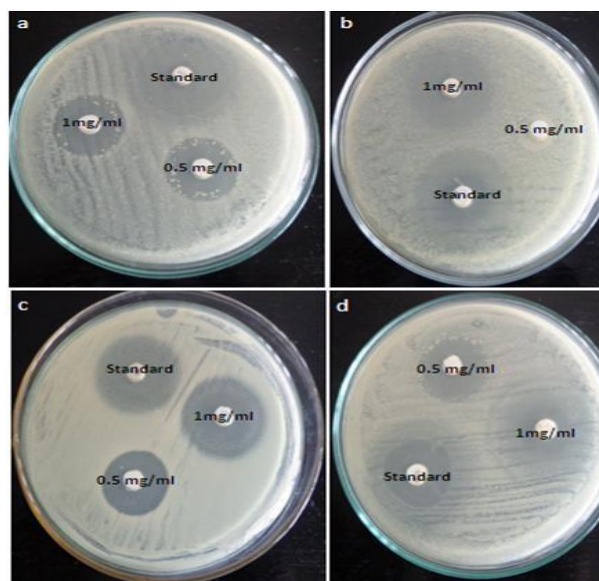
#### 3.1. Antibacterial activity of LA-NPs

The sol-gel route synthesized LA-NPs were tested against clinically isolated both gram positive and gram negative human pathogenic microorganisms. The zone of inhibition was measured for both standard and LA-NPs coated filter paper and the results depicted in figure 1. It was found that LA-NPs coated filter paper disk shown maximum activity against gram positive organisms compared with gram negative organisms. At the concentration of 0.5 and 1mg/ml LA-NPs impregnated filter paper disk achieved maximum activity around 16 and 18 mm against *S. aureus* respectively. For LA NPs impregnated filter paper disk shown significant activity against gram negative bacteria *E. coli* around 15 and 16 mm for 0.5 and 1 mg/ml concentration.



**Fig. 1. Antibacterial activity of LA-NPs against pathogenic bacterium**

LA-NPs impregnated filter paper disk shown significant antibacterial properties against various pathogens investigated and were compared with control. The diameter of inhibition zones increased for the test pathogen (Fig. 2). Whereas, other two clinically isolated bacteria strains of *K. pneumoniae* and *S. pyogenes* showed zone of inhibition of 9 and 12 mm at the concentration of 0.5 mg/ml and 13 and 14 mm at the concentration of 1mg/ml respectively. Whereas, standard antibiotic disk Chloramphenicol obtained 18, 19, 18 and 14 mm against *E. coli*, *K. pneumoniae*, *S. aureus* and *S. pyogenes* respectively. The sol-gel route synthesized LA-NPs showed inhibition zone against all the studied bacteria and we found that the synthesized LA-NPs have good antibacterial action against both gram positive and gram negative bacteria.

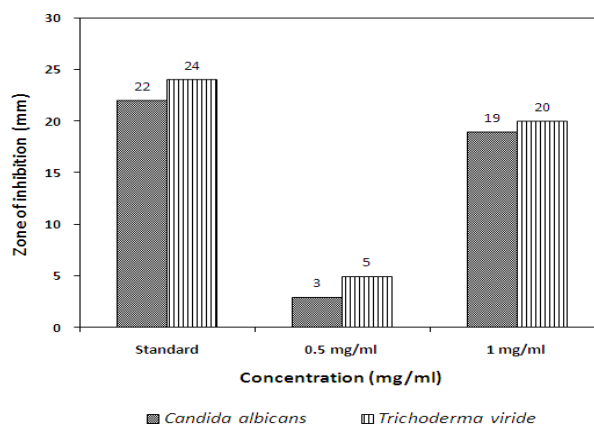


**Fig. 2. Antibacterial activity of LA-NPs against human pathogenic**

a) *E. coli* b) *K. pneumoniae* c) *S. aureus* and d) *S. pyogenes*

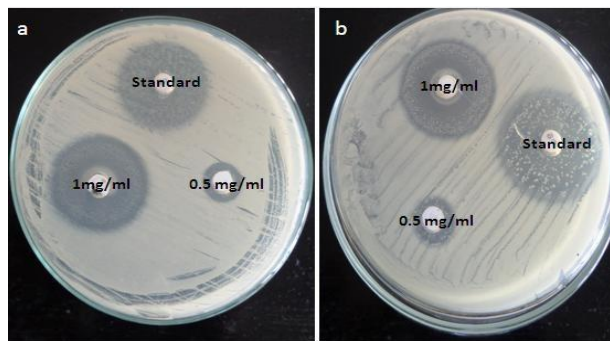
#### 3.2. Antifungal activity of LA-NPs

The sol-gel route synthesized LA-NPs were tested against clinically isolated human pathogenic fungus by standard disk diffusion method. The zone of inhibition was measured for both standard and lanthanum aluminate coated filter paper and the zone of inhibition was shown in figure 3. It was found that lanthanum aluminate coated filter paper disk shown maximum activity against *T. viride* compared with *C. albicans*. At the concentration of 0.5 and 1mg/ml lanthanum aluminate impregnated filter paper disk achieved maximum activity around 5 and 20 mm against *T. viride* respectively. Whereas, lanthanum aluminate impregnated filter paper disk shown significant activity against *C. albicans* around 3 and 19 mm for 0.5 and 1 mg/ml concentration.



**Fig. 3. Antifungal activity of LA-NPs against human pathogenic fungi**

LA-NPs impregnated filter paper disk shown significant antifungal properties against test pathogens investigated and were compared with control. The diameter of inhibition zones increased for the test pathogen at the maximum concentration of 1mg/ml. whereas, standard antibiotic disk Chloramphenicol obtained 22, and 24 mm against *C. albicans* and *T. viride* respectively (Fig. 4). The present experiment clearly revealed that the sol-gel route synthesized lanthanum aluminate showed inhibition zone against the studied human pathogenic fungi and we found that the synthesized lanthanum aluminate have good antifungal action.



**Fig. 4. Antifungal activity of LA-NPs against human pathogenic**

In general, nanoparticles have the ability to anchor to the microorganisms such as bacteria or fungi cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of 'pits' on the cell surface, and there is accumulation of the nanoparticles on the cell surface (Sondi *et al.*, 2004). Developing novel antibacterial agents against bacteria strains, mostly major food pathogens, such as *Escherichia coli* O157: H, *Campylobacter jejuni*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Salmonella*, and *Clostridium perfringens*, has become utmost demand (Hafez *et al.*, 2014). The inhibition of microorganisms growth reported in this present study is dependent on the concentration of LA-NPs in the disk.

Nano-sized particles exhibit varying morphologies and show significant antibacterial activity over a wide spectrum of bacterial species explored by a large body of researchers (Buzea *et al.*, 2007). The exact mechanism which nanoparticles employ to cause antimicrobial effect is not clearly known. However, various theories have been proposed on the action of nanoparticles on microbes to cause the microbial effect against human pathogenic bacteria and fungi.

#### 4. CONCLUSION

In the present study, sol-gel route synthesized LA-NPs impregnated filter paper disk achieved maximum activity against *S. aureus* compared with other three tested organisms such as *S. pyogenes*, *E. coli* and *K. pneumonia*. The synthesized LA- NPs showed very good antimicrobial properties against studied fungi. At the concentration 1 mg/ml LA-NPs impregnated filter paper disk achieved maximum activity against human pathogen.

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