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Biosynthesis of Lanthanum Nanoparticles using Green Gram Seeds and their Effect on Microorganisms.

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ABSTRACT

The application of plant extracts in synthesis of metal nanoparticles commences to be an eco-friendly process. The study deals with synthesis of lanthanum nanoparticles using the extract of *Vigna radiata* (moong, green gram) beans. Green gram extract was prepared to synthesize the nanoparticles. Nanoparticles were characterized using Fourier transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) analysis. Antimicrobial activity of the biologically synthesized nanoparticles was tested by well diffusion technique against nine clinical pathogens. Antioxidant and anticancer studies were performed by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH)method and MTT[3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay against osteosarcoma Mg 63 cell lines respectively with the synthesized lanthanum nanoparticles to check its effectivity and future aspects of the nanoparticles. The antioxidant nature and proliferation of osteosarcoma cell lines confirms that the nanoparticles can be used in future research for anticancer drug development studies. This simple and eco-friendly approach of synthesizing nanoparticles is applicable for large scale synthesis.

Keywords: *Vigna radiata*, lanthanum nanoparticles, FTIR, SEM, DPPH, MTT

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INTRODUCTION

The significant role of nanoparticles in the field of nanochemistry is because of the numerous features that they possess in their tiny size. The efficacy of nanoparticles depends on the size and shape as well as the process of synthesizing the particular nanoparticle [1]. The size of nanoparticles varies within a range of 1-100 nm. The large surface area of nanoparticles makes them suitable for being better sorbents [2].The utilization of nanoparticles depends largely on the process of preparation of the nanoparticle [3]. Biology and medical sciences are related to nanotechnology for more than 50 years. However, in recent years the applications of nanoparticles in clinical analysis and therapeutics have increased many folds [4]. Nanoparticles have gained importance in applications of both *in vitro* and *in vivo* experiments. Biosensing and bioimaging are the two advantageous applications of nanoparticles. The usage of nanoparticles in ocular drugs proved to be a great boon to patients. Nanoparticles deliver the drugs to the specific sites where the drug administration is required [5]. The approach of nanoparticle usage ranges from medical uses to daily uses. In medical uses, nanoparticles are used in treatment of cancer as anticancer drugs, antimicrobial agents, and support system for drug delivery [6]. Lanthanum nanoparticle can be used in optical sensing system. These systems are used in examining the human body temperature. Moreover, lanthanum is capable of inhibiting growth of certain microbes which has been an advantage for lanthanum nanoparticles being used in drugs [7].

The synthesis of metal nanoparticles biologically rather than physical or chemical synthesis have been approached as a better way of protecting environment by many researchers. Among microbes and plants, plants are considered beneficial due to less maintenance processes in plants than bacterial or fungal cultures [8]. Plant products are easily available which helps in biosynthesis of metal nanoparticles in a cost effective method when compared with physical and chemical synthesis. Various unique plants with valuable importance are used in biosynthesis of nanoparticles. Green tea, neem and aloe vera plant extracts are common sources of preparing silver nanoparticles. Natural rubber extract and lemon grass extracts are also used for the same [9]. *Vigna radiata* (green gram) beans have been previously reported in synthesis of silver nanoparticles [10]. No work has been done with *Vigna radiata* seeds extract on synthesis of lanthanum nanoparticles.

Vigna radiata beans are rich source of vitamins, minerals and proteins which is beneficial for human health. Green gram seeds are ideal for patients with diabetes which has a positive effect on the blood glycemic index. The beans as well as the hulls are observed to have an antioxidant nature [11]. The role of green gram beans and its importance in daily human life drives its necessity for using green gram in synthesizing lanthanum nanoparticles which would be further utilized in various drugs and other uses. In this study, green gram extract has been prepared to synthesize lanthanum nanoparticles and was characterized by FTIR and SEM analysis. The antimicrobial activity of the nanoparticles was checked against clinical pathogens [12]. Antioxidant and cytotoxic assay has been performed to check the effectivity of the lanthanum nanoparticles synthesized using green gram beans.

MATERIALS AND METHODS

Collection of the Sample

The green gram seeds were purchased from Vellore market, Vellore, Tamil Nadu.

Chemicals used

All chemicals used including titanium oxide was purchased from Sigma Aldrich.

Collection of bacterial pathogens

Twelve clinical pathogens were collected from Microbial Biotechnology Laboratory, VIT University, Vellore. The pathogens included *Escherichia coli*, *Staphylococcus aureus*, *Serratia marcecens*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Proteus mirabilis* and *Shigella* sp. All the pathogens were used for testing the antimicrobial activity of the titanium oxide nanoparticles synthesized using *Vigna radiata*.

Preparation of the Green Gram Extract

The fresh green gram seeds were washed thoroughly and dried under shade at room temperature. After drying, the seeds were washed again and crushed to small pieces. 10 g of crushed seeds was boiled in 100 ml of distilled water for 30 min and cooled to room temperature. The filtrate was obtained and used as extract for the biosynthesis of lanthanum nanoparticles [13].

Biosynthesis of Lanthanum Nanoparticles using Green Gram Extract

Lanthanum nitrate (0.1 mM) was freshly prepared and kept in stirring condition for 2 h. To 80 ml of the lanthanum nitrate solution, 20 ml of the green gram extract was added and mixed well followed by incubation at room temperature at rotary shaker for 24 h. Following incubation the filtrate was dried in hot air oven. The particles were further powdered and characterized using FTIR and SEM.

Antimicrobial Activity of the Lanthanum nanoparticle

Well diffusion technique was performed to study the effect of the synthesized nanoparticle on clinical pathogens. Pure cultures of the pathogens were freshly inoculated on Mueller Hinton Broth and incubated at 200 rpm overnight. Mueller Hinton agar plates were prepared and each plate was swabbed with individual pathogenic cultures. Wells were cut on the agar plates and 50 µl, 75 µl and 100 µl of the dissolved nanoparticle was added in each well. The plates were incubated at 37°C for 24-48 h and observed for zone of inhibition [14].

Antioxidant Assay

The antioxidant activity of the synthesized lanthanum nanoparticle was checked by DPPH assay. DPPH solution was prepared using methanol. 100 µl of DPPH solution and 100 µl of lanthanum nanoparticle were added to 2.8 ml acetate buffer followed by incubating in dark for 15-20 min. The change in colour was observed and absorbance was measured at 517 nm. Ascorbic acid was taken as standard [15].

$$\text{DPPH Scavenging Percentage} = \frac{\text{Absorbance (Control)} - \text{Absorbance (sample)} \times 100}{\text{Absorbance (Control)}}$$

Cytotoxicity Assay of the Lanthanum nanoparticles

The cytotoxic property of the lanthanum nanoparticles was determined against osteocarcoma cell lines (Mg 63) by MTT assay [16]. The cell lines were obtained from National Centre for Cell Science, Pune, further grown in Eagles Minimum Essential Medium containing 10% FBS. The cells were maintained at 37°C, 5% CO_2, 95% air and 100% humidity. The cell dilution was done in a medium containing 5% FBS until the density reaches $1X10^5$ cells/ml. 100µl cell suspension was added to each well of a 96 well plate at a plating density of 10000 cells/well. The cells were incubated at optimum parameters mentioned above. After 24 h, the cells were treated with serial concentrations of the test samples. DMSO was used for dissolving of sample initially and the nanoparticles were dispersed in PBS. After adding the sample, plates were incubated for 48 hours. Control was maintained without sample.

15µl of MTT in PBS was added in each well and kept for incubation at 37°C for 4 hours. The medium with MTT was removed and formed formazan crystals were solubilized in 100µl of DMSO. The absorbance was measured at 570 nm [17].

$$\text{Percentage of Cell Viability} = (A/A_0) \text{ X } 100$$

Where, A = Absorbance of sample
A_0 = Absorbance of control

Nonlinear regression graph was plotted between percentage of Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software.

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RESULTS AND DISCUSSION

Biosynthesis of Lanthanum nanoparticle and Characterization

The lanthanum nanoparticles were dried and crushed to powder. The crushed powdered particles were used for further characterization.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis has been done within a range of 500 cm^{-1} to 4000 cm^{-1} wave number. The presence of peaks at 3230.77 and 2926.01 depicts C-H stretch and O-H stretch respectively. The presence of nitro compounds are indicated by the peaks in 1548.91 and 1533.41. Peak at 1041.56 and 995.27 shows the existence of C-N stretch and =C-H bend in the sample. Absorption band at 607.58 indicates the lanthanum stretching [18]. The presence of the bands at the above mentioned peaks confirms the synthesis of lanthanum nanoparticles.

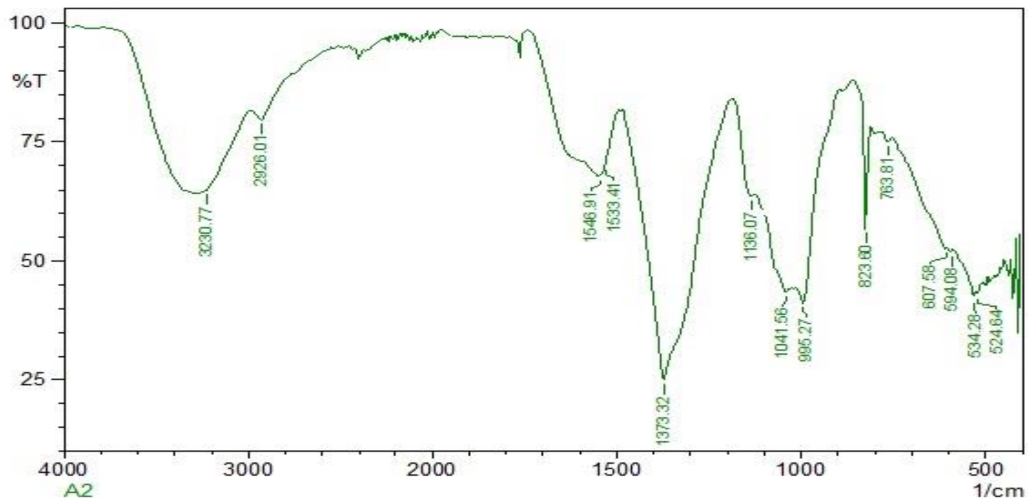

Figure 1: FTIR spectrum of lanthanum nanoparticle synthesized using green gram seeds extract.

Scanning Electron Microscopy

The nanoparticles synthesized were observed for SEM analysis. The particles were round shaped. Similar result was recorded by Balusamy et al. when observed lanthanum nanoparticles under SEM [19]. The particles were found to be present in an aggregated manner.

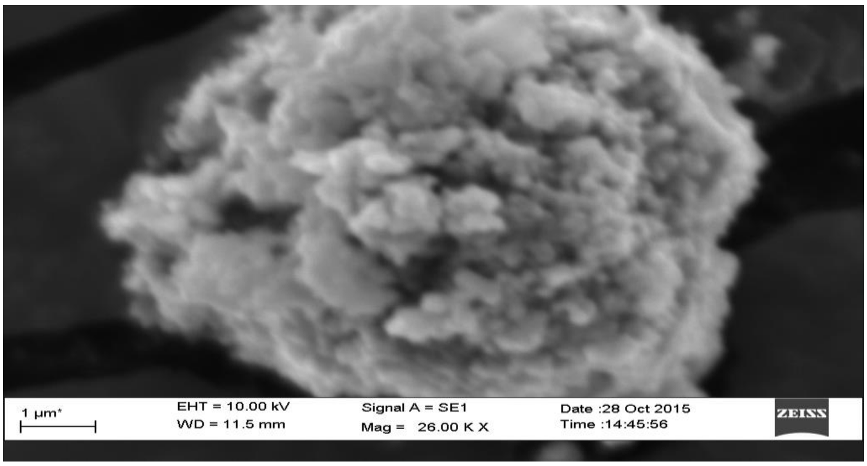

Figure 2: SEM image of lanthanum nanoparticle biologically synthesized using green gram seeds extract

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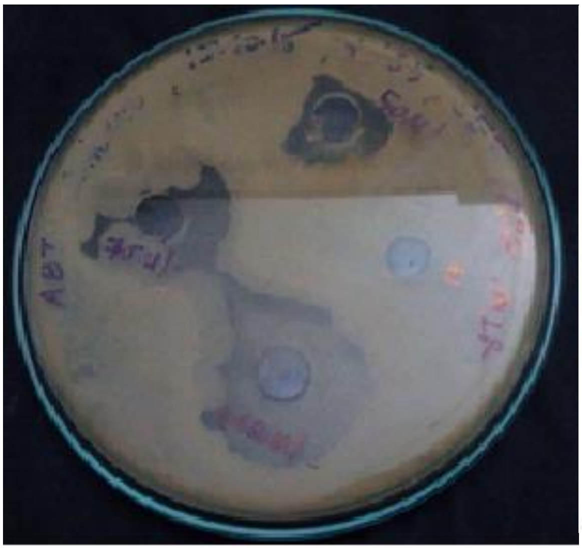
Antimicrobial Activity of Lanthanum Nanoparticles

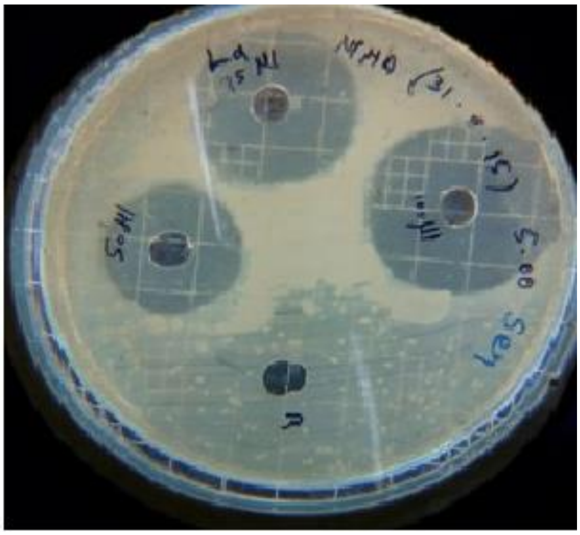
The activity of the biosynthesized lanthanum nanoparticles were checked against nine clinical pathogens. It was observed that the nanoparticles were able to inhibit almost growth of all the bacterial pathogens.

S.NO	ORGANISMS	ZONE OF INHIBITION			
		50 µl	**75 µl**	**100 µl**	**CONTROL**
1.	*Pseudomonas aeruginosa*	1.8 cm	2.4 cm	2.5 cm	-
2.	*Serratia marcescens*	2.4 cm	2.8 cm	3.3 cm	-
3.	*Escherichia coli*	2.5 cm	2.7 cm	2.9 cm	-
4.	*Shigella* sp.	2.5 cm	3.2 cm	3.3 cm	-
5.	*Staphylococcus aureus*	1.3 cm	2.1 cm	2.5 cm	-
6.	*Proteus mirabilis*	1.4 cm	2.0 cm	2.3 cm	-
7.	*Enterobacter* sp.	1.9 cm	2.3 cm	2.8 cm	-
8.	*Salmonella* sp.	1.6 cm	2.4 cm	2.6 cm	-
9.	*Klebsiella pneumonia*	1.7 cm	2.0 cm	2.2 cm	-

Table 1: Antibacterial activity of Lanthanum Nanoparticles biosynthesized using green gram seeds extract against bacterial pathogens



(a)



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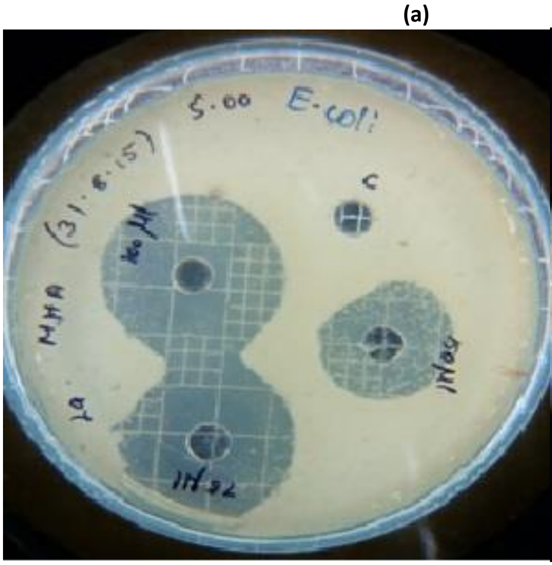

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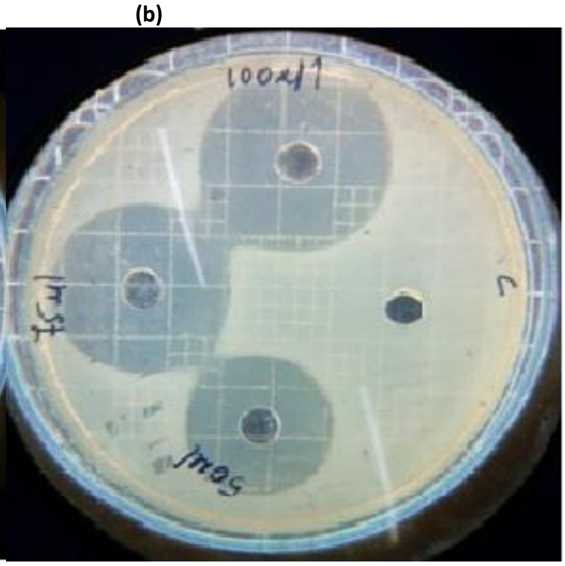

(d)

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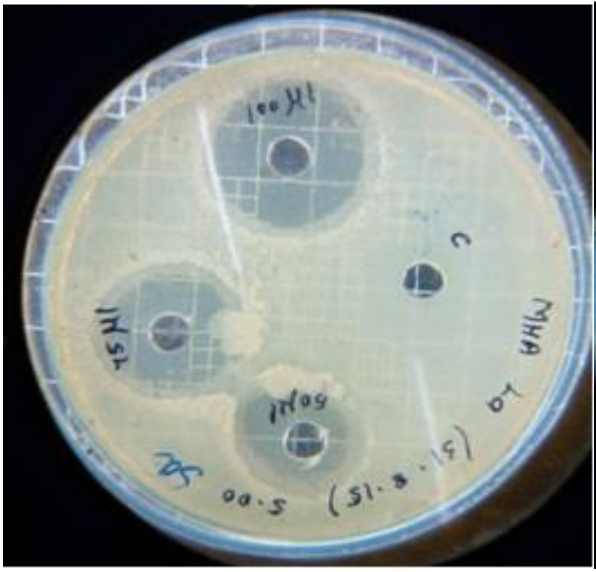

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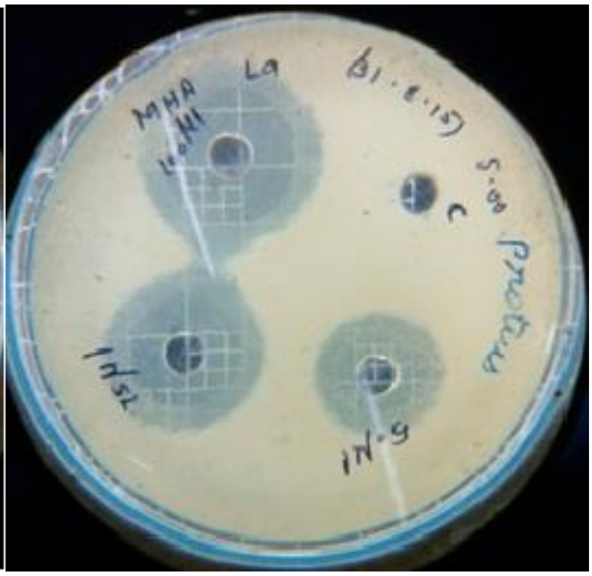

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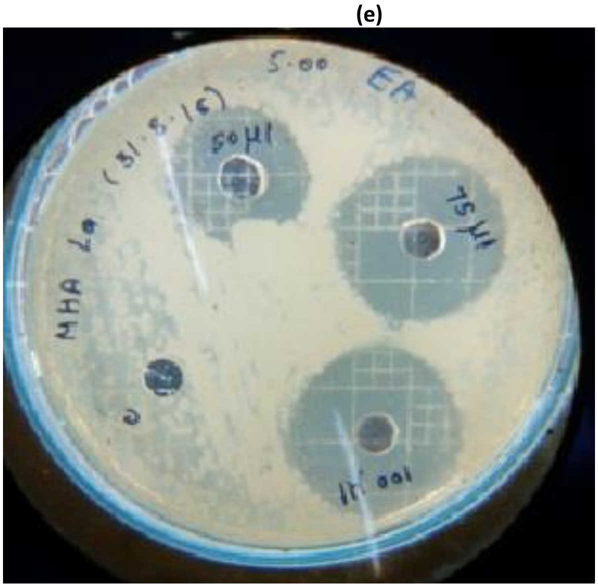

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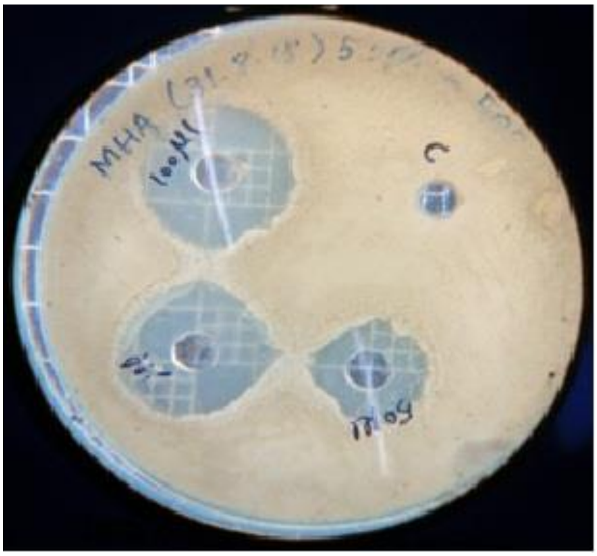

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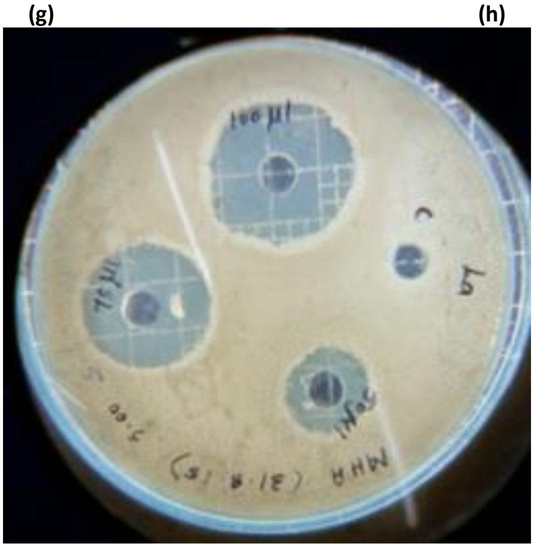


(i)

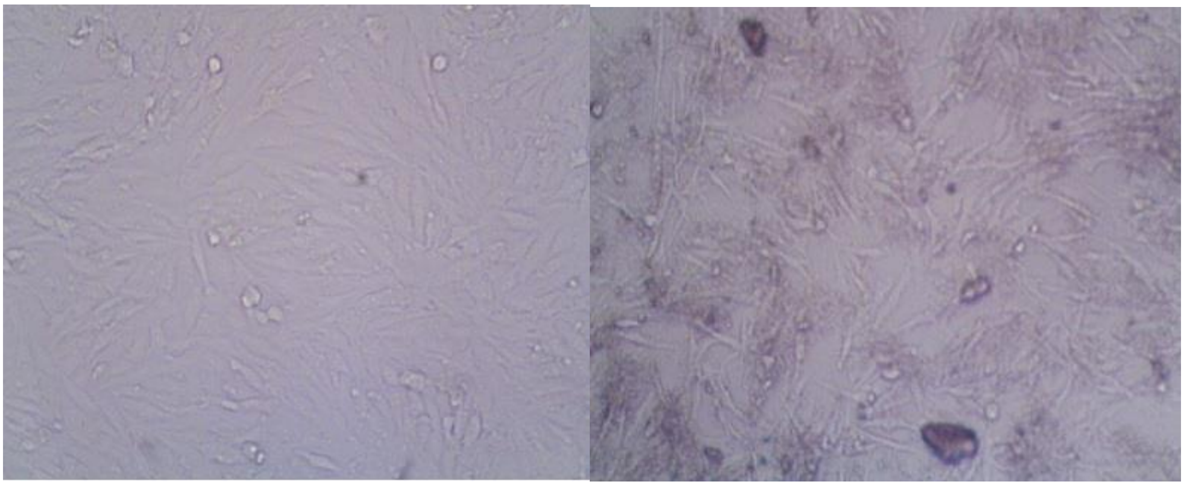
Figure 3: Antibacterial Effect of lanthanum nanoparticles on clinical pathogens: (a): *Pseudomonas aeruginosa*, (b): *Serratia marcescens*, (c): *Escherichia coli*, (d): *Shigella* sp., (e): *Staphylococcus aureus*, (f): *Proteus mirabilis*, (g): *Enterobacter*sp., (h): *Salmonella* sp., (i):*Klebsiella pneumonia*.

Antioxidant Activity of the Lanthanum Nanoparticles

The colour change in the test sample after 15 min incubation determines the nanoparticles to be antioxidant. The absorbance of the sample was measured to calculate the percentage of the antioxidant nature of the biosynthesized nanoparticles. The antioxidant nature increases with the increase in lanthanum nanoparticles concentration. The result proves that the lanthanum nanoparticle synthesized from the green gram shows antioxidant nature and can act against free radicals [20].

Cytotoxicity Activity

The lanthanum nanoparticles synthesized using green gram showed cytotoxic activity against osteosarcoma cell lines. At IC50 value of 200 µg/ml, the proliferation of osteosarcoma cells (Mg 63) was inhibited significantly. In previous studies, zinc and silver nanoparticles were found to be cytotoxic against Mg 63 cell lines [21]. However, no reports have been found on cytotoxic effect of lanthanum nanoparticles on Mg 63 cell lines.



(a) **(b)**

Figure 4: Cytotoxicity activity of the lanthanum nanoparticles: a: Control, (b): Test

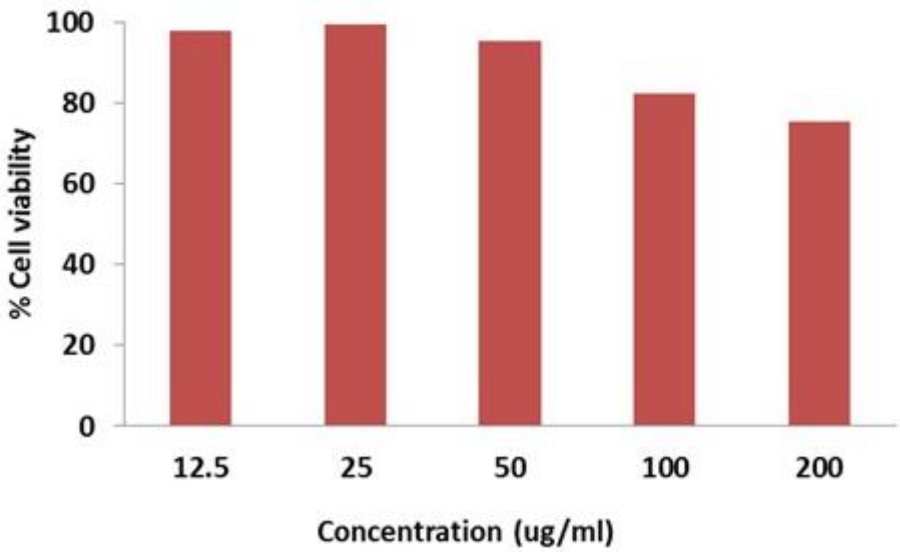


Figure 5: Graph showing cell viability of Mg63 cell lines when treated with biosynthesized lanthanum nanoparticles

CONCLUSION

Lanthanum nanoparticles were biosynthesized using green gram beans and characterized by FTIR and SEM. Presence of different functional group and lanthanum stretch confirmed the existence of lanthanum nanoparticles. The biosynthesized lanthanum nanoparticles were effective against nine pathogenic bacteria and showed significant antioxidant nature. Proliferation of Mg 63 cell lines was inhibited by the nanoparticles which indicate that the particles can be further used in drug development.

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