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Green synthesis of Linezolid conjugated silver nanoparticles from Clerodendrum chinense extract, a medicinal plant and their antimicrobial effect on MDR bacteria

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ABSTRACT:
Multidrug resistant (MDR) microbes which show resistance against typical antibiotics is a global problem. Various strategies are being employed against MDR pathogens. In the present study, silver nanoparticles (AgNPs) and Linezolid conjugated silver nanoparticles (Li-AgNPs) were synthesized using Clerodendrum chinense leaf extract. The synthesized nanoparticles were characterized by different analytical techniques to establish the formation, size, shape and the presence of functional groups. The UV-visible spectra with absorption bands at 406 nm and 418 nm confirm the formation of AgNPs and Li-AgNPs. The SEM images showed that the synthesized AgNPs and Li-AgNPs were found to be spherical in shape with a diameter ranging from 16 to 20 nm and 22 to 26 nm, respectively. The X-ray diffraction pattern of AgNPs and Li-AgNPs showed peaks establishing the crystalline nature with characteristic peaks of 2θ for (111), (200), (220) and (311) planes. The FTIR spectrum of AgNPs showed absorption bands for different chemical groups and Li-AgNPs showed absorption peaks corresponding to both AgNPs and Linezolid. Antimicrobial studies against Enterococcus faecium and Staphylococcus aureus MDR strains indicated that Li-AgNPs showed 46.6% and 52.38% more growth inhibition compare to linezolid, respectively. These findings imply the possible use of Li-AgNPs against MDR pathogens.

Keywords: Clerodendrum chinense, linezolid, Enterococcus faecium, Staphylococcus aureus, Silver nanoparticles.

I. INTRODUCTION

Glory bower (Clerodendrum chinense) is a flowering plant belonging to the genus Clerodendrum. This plant is native to Nepal, Assam, the Eastern Himalayas, the Andaman and Nicobar Islands, south-central and south-east China, south-east Asia, and Malaysia [1, 2]. It is a perennial shrub that grows up to a length of 3m [3].The roots and leaves of C. chinense have been used in traditional medicine for the treatment of rheumatism, asthma, and inflammatory diseases [4].

Linezolid belongs to the class antibiotics called oxazolidinones. It is used for the treatment of infections caused by Gram-positive bacteria that are resistant to other antibiotics [5]. It is used to treat infections including pneumonia and skin infections. It works by stopping the bacterial growth. Linezolid has demonstrated that it binds to a deep cleft of the 50s ribosomal subunit that is surrounded by 23s rRNA nucleotides. Mutation of 23s rRNA was shown to be a linezolid resistance mechanism [6].

The word nano means 10^{-9} or one billionth of a meter. The term “nano” is derived from a Greek word means “dwarf”. Nanotechnology deals with the application of nanoparticles in biological, chemical, physical, environmental, agricultural, industrial or pharmaceutical sciences [7]. Nanoparticle is a small particle ranges between 1-100nm[8]. Silver nanoparticles are emerging as one of the fastest growing materials due to their distinct properties, small size and high surface area [9]. Nanobiotechnology is the combination of engineering and molecular biology which is leading to a new set of multifunctional devices and systems for biological and chemical analysis with greater sensitivity, specificity and higher rate of recognition[10]. Analysis of signalling pathways by nanobiotechnology techniques might provide new insights into disease processes, thus identifying more efficient biomarkers and understanding the mechanisms of drug action [11]. Multi drug resistant (MDR) bacteria are those which are resistant to currently used antibiotics. In the present study, the antimicrobial efficacy of silver nanoparticles was assessed against two MDR strains Enterococcus faecium and Staphylococcus aureus. The Enterococcus faecium is a gram positive gamma-haemolytic or non-hemolytic bacterium belonging to the genus Enterococcus. It is found in humans and animals gastrointestinal tract, but it may be pathogenic, causing diseases such as neonatal meningitis or endocarditis [12]. Staphylococcus aureus is a Gram-positive spherically shaped bacterium, normally found in the upper respiratory tract and on the skin. It is a facultative anaerobe that can grow in absence oxygen [13]. It can also be an opportunistic pathogen, causing skin infections including abscesses, respiratory infections such as sinusitis and food poisoning. S. aureus is one of the leading pathogens for deaths associated with antimicrobial resistance and the emergence of antibiotic-resistant strains, such as Methicillin-resistant S. aureus (MRSA), is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for S. aureus has been approved.

II. MATERIALS AND METHODS:

Sample collection and extract preparation:

Clerodendrum chinense plant was collected from Shankaraghatta. About 30 gm of fresh and healthy leaves were boiled with 300 ml double distilled water in 500 ml beaker for 30 to 40 minutes. The extract was cooled and filtered through Whatman no.1 filter paper to get clear solution. The filter was refrigerated in 250ml Erlenmeyer flask at 4⁰C for further experimental use [14].

Biosynthesis of silver nanoparticles (AgNPs)

The silver nitrate solution (1mM) was mixed with 100ml of prechilled Clerodendrum chinense leaf extract in 1:1 ratio drop by drop. The mixture was thoroughly mixed with vigorous stirring on a magnetic stirrer. The mixture turns from greenish yellow to brown. The UV visible spectrophotometer reading was measured to monitor the synthesis of AgNPs [15].

Synthesis of Linezolid conjugated silver nanoparticles (LI-AgNPs)

The mixture of bactericide (Linezolid 2%) and Clerodendrum chinense plant extract was prechilled for 15 min. Silver nitrate solution was added to the mixture drop by drop with vigorous stirring on a magnetic stirrer for about 5–10 min until the colour of the mixture turns from greenish yellow to yellow and yellow to dark brown [16, 17, 18].

Characterization of silver nanoparticles

UV – visible spectroscopy

The optical properties of synthesized AgNPs and Li-AgNPs were determined by using UV Spectroscopy in the range 320 to 450nm. The nanoparticles have characteristic absorption maxima between 350 to 450nm [19].

Scanning electron microscopy (SEM):

The SEM analysis is the best method for determining the surface topography and 3D view of the synthesized nanoparticles. The morphological characteristics of AgNPs and Li-AgNPs were established by SEM. Thin films of the samples were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the SEM grid and the film was allowed to dry by keeping it under a mercury lamp for 5 min and then subjected for SEM analysis [20].

X-ray Diffraction

X-ray diffraction (XRD) is a popular analytical technique which is used for the analysis of both molecular and crystal structures. The qualitative identification of various compounds, quantitative resolution of chemical species, measurement of degree of crystallanity, isomorphous substitutions, particle sizes etc. can be obtained by XRD. When X-ray light reflects on any crystal, it leads to the formation of many diffraction patterns and the patterns reflect the physio-chemical characteristics of the crystal structures. In a powder specimen, diffracted beams typically come from the sample and reflect its structural physico-chemical features[21].

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy is able to provide accuracy, reproducibility and also a favorable signal to noise ratio. By using FTIR spectroscopy, it becomes possible to detect small absorbance changes which help to perform difference spectroscopy, where one could distinguish the small absorption bands of functionally active residues from the large background absorption. FTIR spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles, which is more pronounced in academic and industrial research. Furthermore, FTIR has also been extended to the study of nano-scaled materials, such as confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, graphene and gold nanoparticles, or interactions occurring between enzyme and substrate during the catalytic process [22].

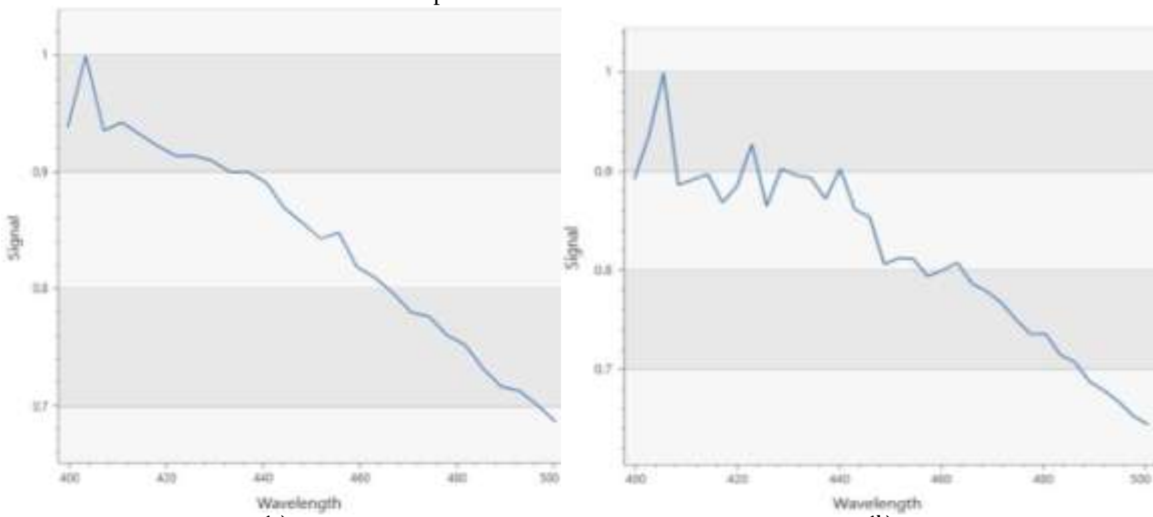
In vitro antimicrobial of AgNPs and Li-AgNPs

Biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against gram- positive Enterococcus faecium and Staphylococcus aureus. Aqueous dispersions of silver nanoparticles in a same concentration (75µl) were made. Stock cultures of E. faecium and S. aureus were grown separately in liquid nutrient broth medium. Inoculated bacterial culture were added to nutrient broth medium and incubated for 24 hours at 37° C. These cultures were spread on the solidified nutrient agar media. AgNps, Li-AgNPs, Antibiotic Linezolid and leaf extracts were added to the wells made in the media. The plates were incubated at 37° C for 24 hours. The antimicrobial effect was observed after 24 hour.

III. RESULT AND DISCUSSION

UV-Visible Spectroscopy

The UV-Visible spectroscopy was used for the structural characterization of synthesized AgNPs. The absorption band in 350 to 550 nm region is typical for the AgNPs. The UV-visible spectra showed absorption bands at 406 nm and 418 nm which confirm the formation of AgNPs and Li-AgNPs.

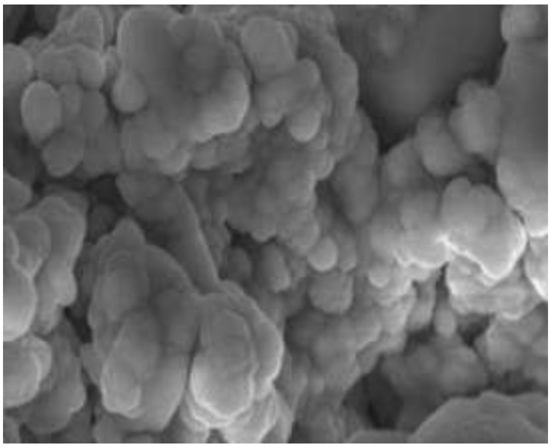


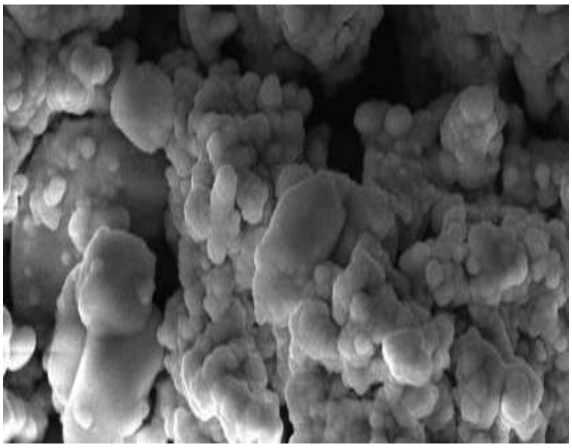
1a) 1b)

Figure 1: UV- Vis absorption spectra of a) AgNPs and b) Li-AgNPs

Scanning electron microscopy (SEM) analysis

Microscopic surface features including morphology and particle size of synthesized AgNPs and Li-AgNPs were assessed by SEM analysis. The nanoparticles were found to be spherical in shape with a diameter ranging from16 to 20nm and 22 to 26 nm, respectively. SEM image also confirms that the synthesized nanoparticles were well separated with no aggregation.





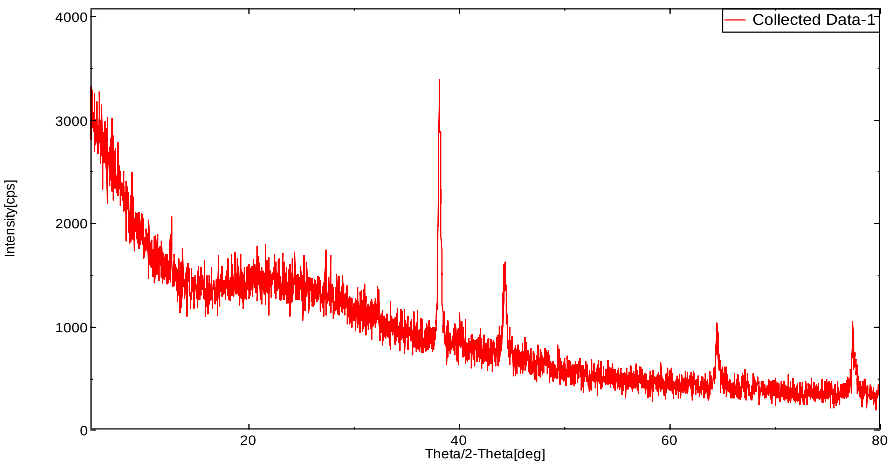
2a) 2b)

Figure 2: SEM Images of a) AgNPs and b) Li-AgNPs

X-ray Diffraction analysis

The synthesized AgNPs and Li-AgNPs from Clerodendrum chinense plant were subjected to X-Ray diffraction studies to understand the crystallanity and to establish the average particle size. As shown in (Fig. 3a), The XRD pattern of AgNPs synthesized from Clerodendrum chinense plant has prominent characteristic peaks of the 2θ at 38.19°, 44.32°, 64.54°, and 77.44° which can be assigned to (100), (33.36), (25.87), and (28.90) planes, respectively, with some minor peaks.

The XRD Pattern of Li-AgNPs (Fig 3b), shows prominent characteristic peaks of 2θ at45.29°,and 75.85° corresponding to AgNPs, and peaks of 2θ at 25.99°, 34.34°, 35.2° corresponding to Linezolid.



a)

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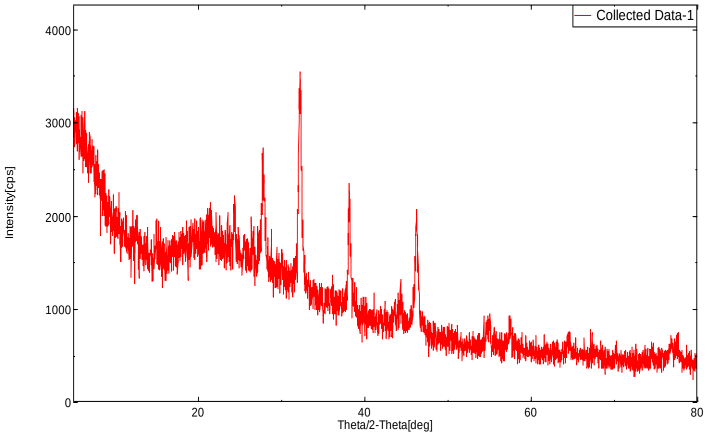


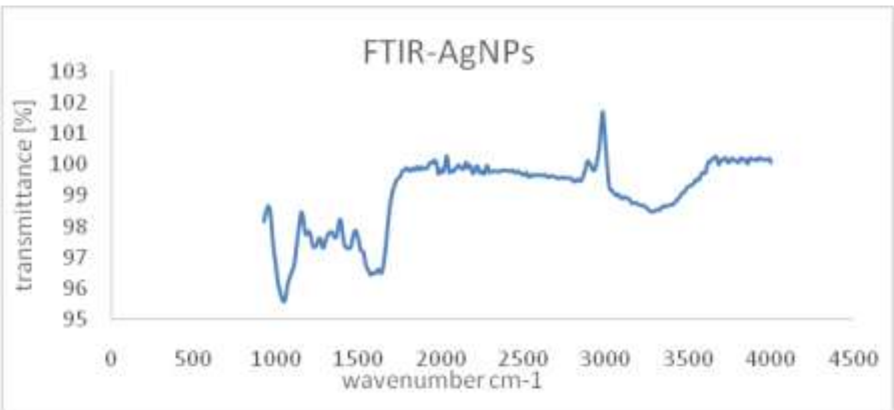
b)

Figure 3: XRD Images of a) AgNPs and b) Li-AgNPs

Fourier Transform Infrared Spectroscopy

The synthesized AgNPs and Li-AgNPs from Clerodendrum chinense plant were subjected to Fourier transform infrared spectroscopy studies. The FTIR spectra of synthesized AgNPs showed various absorption bands for different chemical groups [Fig 4a] 1573.94cm^{-1} showed the stretching vibration of primary amine -N-H group and 1046.32 cm^{-1} corresponds to C-O Primary alcohol group at 95.57% and 96.63% respectively.

The FTIR spectrum of Li-AgNPs synthesized from Clerodendrum chinense plant [Fig4 b] shows distinct peaks at1014.98cm^{-1} at 99.26% illustrating the -C-O- alcohol group which confirms the AgNPs. The peaks at 3739.23cm^{-1}, 2166.89cm^{-1}and 1303.95cm^{-1}, at 97.52%, 98.64%, 99.64% establish the adhesion of Linezolid on the AgNPs.



a)

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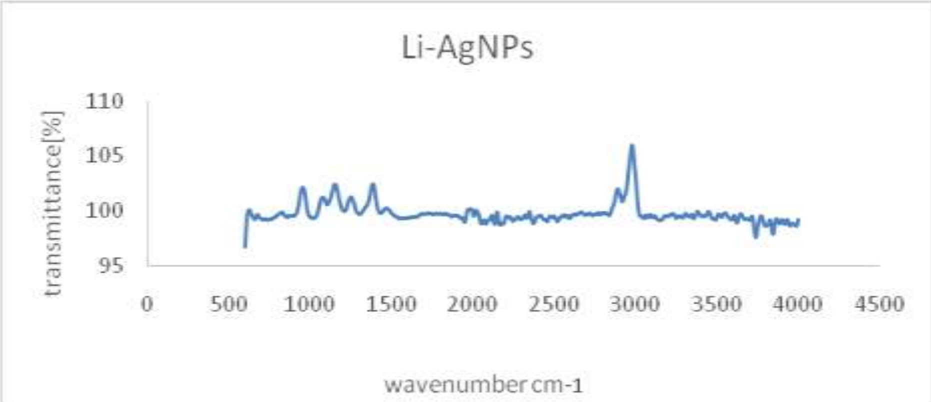

b)

Figure 4: FTIR Images of a) AgNPs and b) Li-AgNPs

Antimicrobial activity of AgNPs and Li-AgNPs

The antimicrobial potential of AgNPs and Li-AgNPs was assessed against E. faecium and S. aureus. The result showed that the inhibition of antimicrobial growth was observed with both AgNPs as well as Li-AgNPs.

In case of E. faecium, the Clerodendrum chinense plant extract showed 25%, AgNPs showed 33.3% and where as the Li-AgNPs showed 46.6% more growth inhibition activity compare to standard antibiotic Linezolid.

In case of S. aureus, the C. chinense plant extract showed 30%, AgNPs showed 37.5% and Li-AgNPs showed 52.38% more growth inhibition activity compare to standard antibiotic linezolid.

Name of microorganisms	*clerodendrum chinense* plant Extract	AgNPs	Li- AgNPs	Linezolid
Enterococcus faecium	**1.0±0.02**	**1.2±0.01**	**1.5±0.03**	**0.8±0.02**
Staphylococcus aureus	**0.6±0.03**	**1.6±0.02**	**2.1±0.01**	**1.0±0.03**

Table 1: The effect of AgNPs and Li-AgNPs on the growth inhibition of Enterococcus faecium, Staphylococcus aureus

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a) b)

Figure 5; Antimicrobial activity of different treatments, (1) Clerodendrum chinense plant extract, (2) silver nanoparticles, (3) linezolid conjugated silver nanoparticles (4) linezolid antibiotic for different microorganisms.

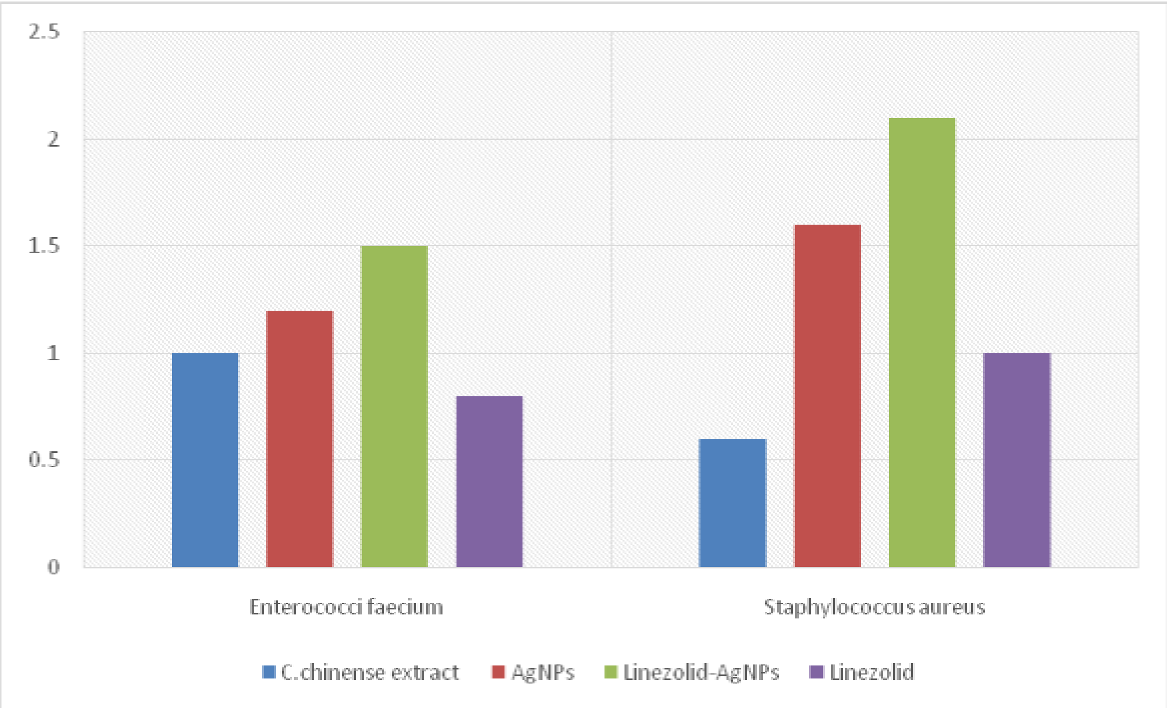

Figure 18: Antimicrobial activity of C. chinense plant extract, AgNPs, Li-AgNPs and standard antibiotic Linezolid against Enterococcus faecium and Staphylococcus aureus.

IV. CONCLUSION:

Nanotechnology deals with the application of nanoparticles in biological, chemical, physical, environmental, agricultural, industrial or pharmaceutical science. Nanoparticle is a small particle range between 1-100 nm. Silver nanoparticles are emerging as one of fastest growing material due to their distinctive physical, chemical and biological properties. Nanotechnology is relatively new and although full scope of contribution to those technological advances in the field of human health care remains unexplored. Nanotechnology will have a deep impact on disease prevention diagnosis and treatments. Silver nanoparticles possess unique properties with a wide range of application such as antimicrobial, anticancer, catalyst and wound healing activities. In this study, AgNPs and Li-AgNPs were synthesized by using C. chinense leaf extract and characterized by various techniques. The formation of AgNPs and Li-AgNPs was confirmed by the characteristic UV-Visible absorption peaks. The surface topography of the synthesized nanoparticles, as analyzed by SEM, indicated the uniform size and shape. FTIR data indicated the nature of functional groups present and structural features of nanoparticles. The Li-AgNPs showed significantly high antibacterial potency against MDR E. faecium and S. aureus as compared to AgNPs and antibiotic linezolid alone.

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Author contribution Author 1: Malini B P: collected the data, designed the analysis and performed the analysis. Author 2: Raghu H S: conceived and designed the analysis and wrote the paper Author 3: Raghavendra S N: designed the analysis Author 4: designed the analysis and manuscript preparation and editing.

Data and code availability The raw/processed data required to produce these fndings cannot be shared at this time due to legal or ethical reasons. The data will be made available on request.

Declarations

Ethical approval Not applicable.

Conflict of interest

The authors have declared no conflict of interest

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