RESEARCH PAPER

Synthesis and characterization of linezolid conjugated silver nanoparticles using *Thespesia Populnea* leaf extract and evaluation of antibacterial efficacy against MDR pathogens: A novel method against MDR bacteria

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ABSTRACT

Objective(s): Multidrug-resistant (MDR) organisms are posing threat by exhibiting resistance to commonly used antibiotics. From our study, we found a novel strategy to enhance the efficacy of antibiotics, which could help in treating diseases caused by various pathogens. We have used a natural, low-cost, biological reducing and capping agent, *Thespesia populnea* leaf extract to synthesize silver nanoparticles (AgNPs) and linezolid-conjugated silver nanoparticles (Li-AgNPs).

Materials and Methods: The nanoparticles were characterized by UV-visible spectroscopy, Scanning Electron Microscope (SEM), X-Ray Diffraction (XRD), and Fourier Transform Infrared (FT-IR) spectroscopy. The antimicrobial activity was evaluated against MDR *Staphylococcus aureus, Enterococcus faecalis*, and *Enterococcus faecium* by agar well plate method. The UV-visible absorption spectra showed absorption peaks at 442 nm and 464 nm for AgNPs and Li-AgNPs, respectively.

Results: The SEM analysis revealed particle size with a diameter ranging from 18 to 22 nm and 24 to 32 nm, respectively, and spherical in shape. The FT-IR spectrum has a distinct absorption band at 2065 cm_{-1} confirms adsorption of antibiotic linezolid on the AgNPs. The XRD pattern showed the characteristic absorption bands of 2 theta values, which confirms that NPs are crystalline in nature. The AgNPs and Li-AgNPs have exhibited the antibacterial potency. The Li-AgNPs have showed 25.8%, 7.6%, and 12.5% more microbial growth inhibition compared to antibiotic linezolid against *E. faecalis, E. faecium*,and *S. aureus*,respectively.

Conclusion: All these results clearly indicated that the Li-AgNPs possesses enhanced antimicrobial activity than antibiotic linezolid, indicating the usefulness of this novel strategy to treat various communicable diseases caused by MDR pathogens.

Keywords: *Communicable diseases, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, Anti-Bacterial Agents, Linezolid*

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INTRODUCTION

The term "Nano" is derived from a Greek word "dwarf". The prefix nano in the word nanotechnology means a billionth ($1/10_{-9}$). "Nanotechnology" deals with application of nanoparticles in biological, physical, chemical, environmental, agricultural, industrial or pharmaceutical sciences [1-4]. In the last few decades, nanotechnology has become an important and exciting forefront field in physics, chemistry, biology and engineering. It shows great promise for providing us with a wide range of applications in the near future, with many breakthroughs. Nanobiotechnology is a newly coined term describing the convergence of the two existing, however distant, worlds between engineering and molecular biology. It is believed that a combination of these disciplines will produce a new class of multifunctional devices and system for biological and chemical analysis characterized by better sensitivity and specificity and higher rates of recognition compared with current solutions [5-9].

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Solid particles or particulate dispersion with a size range of 10-100 nm are considered as nanoparticles. The nanoparticles are synthesized by various methods, in which biosynthesis or green synthesis is an environmental friendly approach, which uses plant extracts, bacteria, fungi, and so on to produce nanoparticles [10-14]. The nanomaterials can be classified based on their number of dimensions. According to Siegel, nanomaterials are classified as zero-dimensional (OD), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) nanomaterials [15-17]. Particularly, silver nanoparticles (AgNPs) have received more attention due to their physical, chemical, and biological properties that are attributed to their catalytic activity and bactericidal effects. They are used as antimicrobial agents in wound dressing, as antiseptic lotion to prevent wound infections, and as anticancer agents [18-20]. Among various classes of nanoparticles, Silver nanoparticles (AgNPs) have received greater attention due to its stability (noble metal) and application as an antimicrobial agent. The antimicrobial potency of silver increases with increased surface area [21-24]. The biological activity of AgNPs depends on number of factors such as their surface chemistry, size, size distribution, particle morphology, shape, particle composition, coating or capping, agglomeration, dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type. One of the most important factors in determining cytotoxicity is the kind of reducing agents utilized in the synthesis of AgNPs [25-30].

Thespesia populnea is a flowering species belonging to the Malvaceae family commonly known as the Portia tree, Pacific rosewood, Indian tulip tree, or Milo. It is a small tree or shrub that can grow up to 60 meters tall with a trunk that can get as big as 20-30 cm in diameter. It grows in regions which are 275 m above the sea level and receives an annual rainfall of 500-1600 mm. The plant parts of *T. populnea* particularly the bark, roots, leaves, flowers, and fruits are all used in Indian traditional medicine against a wide range of conditions like pleurisy, cholera, colic fever, herpes, urinary track problems, abdominal swellings, hair lice, swollen testicles, rheumatism, cough, influenza, headaches, skin diseases, hemorrhoids, cold, etc. Apart from these medicinal paper are, its timber is also used to make the paper. Its wood is used to make bowls, paddles, and other carved objects [31]. In the present study, we have prepared linezolid conjugated silver nanoparticles (Li-AgNPs) using aqueous leaf extract of *Thespesia populnea* and evaluated its antibacterial effect against MDR *Staphylococcus aureus, Enterococcus faecalis*, and *Enterococcus faecium*. This novel strategy could be employed as an effective strategy for treating various other communicable diseases as well. In addition, this method is eco-friendly, non hazardous and economical.

MATERIALS AND METHODS

Preparation of Thespesia populnea leaf extract

Fresh and healthy leaves of *T. populnea* were collected from Honnenalli, Chitradurga district, Karnataka, India. About 30 g of healthy leaves were washed thoroughly and boiled with 300ml of distilled water for 30-40 min. The obtained extract was cooled and filtered twice through Whatman no.1 filter paper to get a clear solution. This extract was stored in refrigerator at 4_0 C for further use [32].

Biosynthesis of silver nanoparticles

The silver nitrate solution (3mM) was mixed with *T. populnea* leaf extract solution in the ratio of 1:2. The mixture was thoroughly mixed and incubated at 37_0 C in dark condition for 24 hr. The colour change from yellowish to dark brown was observed, mainly due to reduction of silver nitrate to metallic silver by the secondary metabolites present in the *T. populnea* leaf extract. These metabolites include alkaloids, terpenoids, and flavonoids which act as reducing and capping agents. The UV-visible absorption was measured in the range of 350-450 nm to confirm the synthesis of AgNPs using UV-visible spectrophotometer. Then the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and pellet was collected. The separated pellet was dried and used for further characterization [33].

Synthesis of Linezolid conjugated AgNPs

The *T. populnea* leaf extract, 3mM $AgNO_3$ solution, and Linezolid (0.1% w/v) were mixed in the ratio of 1:3:1. The mixture was thoroughly mixed and incubated at 370 C in dark condition for 24 h. The change in the colour of the mixture was observed. The UV-visible absorption was measured in the range of 350-450nm to confirm the synthesis of Li-AgNPs by UV-visible spectrophotometer. Then the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and pellet was collected. The separated pellet was dried and used

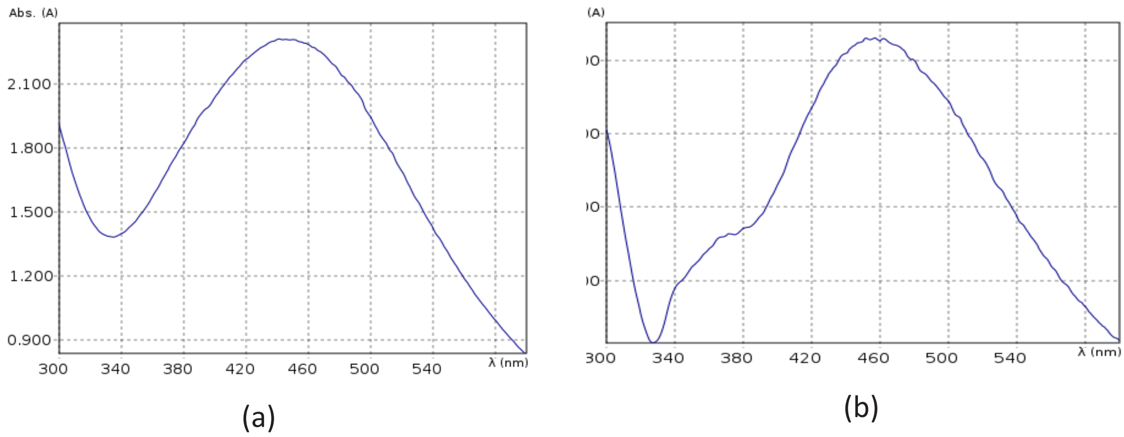


Fig 1. UV-visible absorption spectra of (a) AgNPs, and (b) Li-AgNPs.

for further characterization [34].

Characterization of silver nanoparticles

UV-visible spectroscopy

The optical properties of synthesized AgNPs were determined by using UV-visible spectrometry. The UV-visible absorption spectra of AgNPs and Li-AgNPs were observed in the range 350 to 450 nm using UV-visible spectrophotometer (Eppendorf).

Scanning electron microscopy (SEM)

The morphological characteristics of AgNPs and Li-AgNPs were established by the SEM as the best method for determining the surface topography and three-dimensional view of the synthesized NPs. 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. The film was allowed to dry by keeping it under a mercury lamp for 5 min and then, was subjected for the SEM analysis [35].

Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR analysis was used to identify the functional groups present in the *T. populnea* leaf extract which may be responsible for the reduction of $AgNO_3$ to metallic silver. These metabolites may be involved in the synthesis and stabilization of AgNPs and Li-AgNPs [36].

X-ray diffraction studies

The sizes of particle and nature of AgNPs were resolved by XRD. A thin film of the dried NPs was coated on an XRD grid and carried out XRD studies. The obtained data of 2 theta values are helpful for analyzing different planes of crystal. The average size of crystalline AgNPs was calculated from the width of the peaks [37].

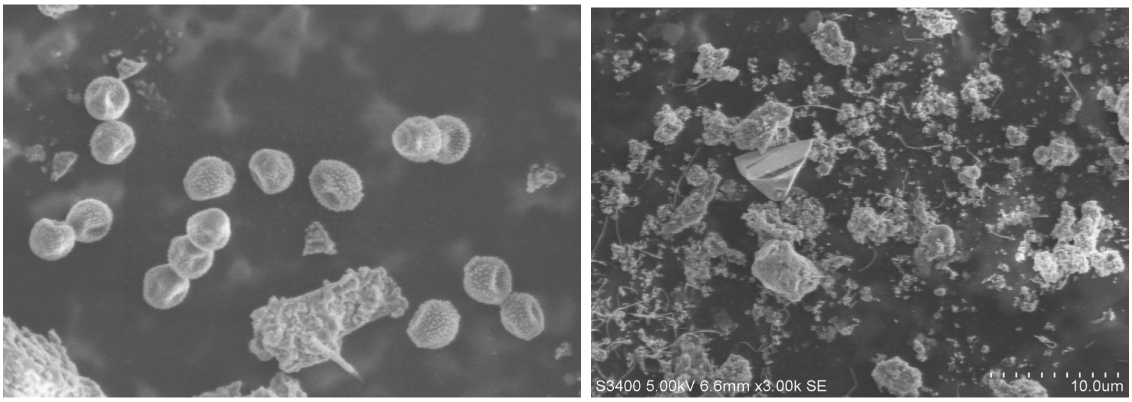
In-Vitro antimicrobial activity of AgNPs and Li-AgNPs

The antimicrobial activity of synthesized AgNPs and Li-AgNPs was assessed against gram positive bacteria *E. faecium, E. faecalis,* and *S. aureus* by agar well plate method. The Aqueous dispersions of AgNPs and Li-AgNPs (0.1%) were used in the same concentration as that of antibiotic linezolid. Stock cultures of *E. faecium*, *E. faecalis,* and *S. aureus* were grown separately in liquid nutrient broth medium. The bacterial cultures were spread uniformly on solidified nutrient agar media and 4 wells of 1cm diameter were made in each plate with sterile cork borer. The *T. populnea* leaf extract, AgNPs, Li-AgNPs, and Linezolid were loaded to the different wells. These Petri plates were incubated at 37 $_0$ C for 24 h for microbial growth [38].

RESULTS AND DISCUSSIONS

UV-visible spectroscopy

The UV- visible spectroscopy was used to measure the UV- visible absorption in the range of 350 to 550 nm which is typical for AgNPs. In the present study, the UV-visible absorption spectra of AgNPs and Li-AgNPs have shown the characteristic absorption peaks at 442 nm and 464 nm, respectively (Fig. 1a & 1b).



(a) (b)

Fig 2. SEM images of (a) AgNPs, and (b) Li-AgNPs

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 from 18 to 22 nm and 24 to 32 nm, respectively (Fig. 2a & 2b). SEM image also confirms that the synthesized nanoparticles were well separated with no aggregation.

FT-IR analysis

FT-IR measurements were carried out to identify functional groups present in the secondary metabolites of *T. populnea* leaf extract responsible for the capping and efficient stabilization of synthesized AgNPs. The FT-IR spectra of AgNPs and Li-AgNPs show several peaks indicating the complex nature of the biological material. The FT-IR spectra of AgNPs shows absorption bands at 3000 cm^{-1},1064 cm^{-1},and 1116 cm^{-1} corresponding to O-H, C=O, and C=C groups, respectively (Fig. 3a). The FT-IR spectra of Li-AgNPs showed the absorption bands at 3720 cm^{-1}, 3000 cm^{-1}, 2065 cm^{-1}, and 1100 cm^{-1} which

(a)

(b)

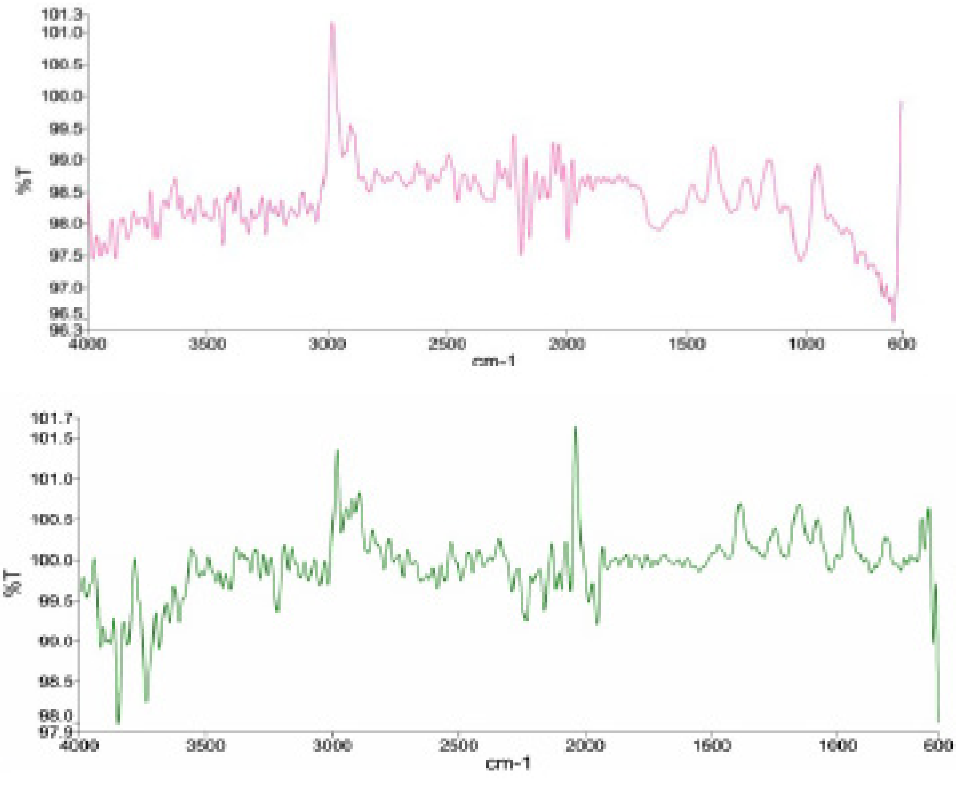
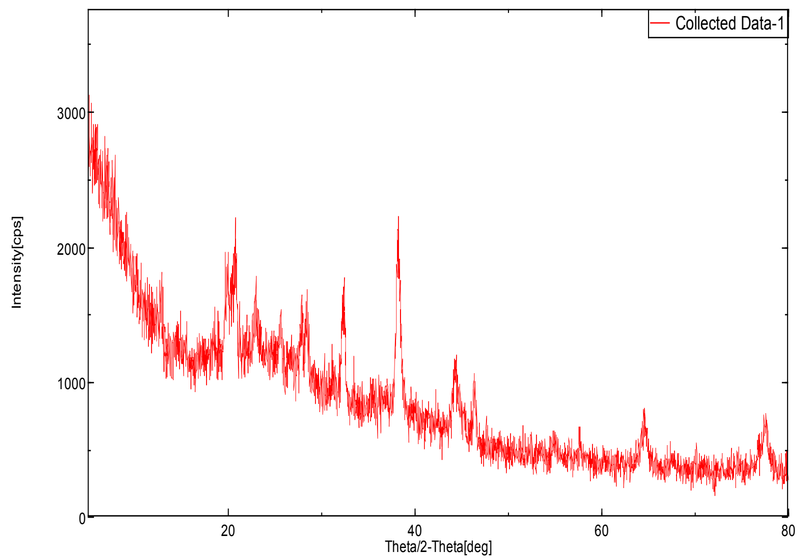


Fig 3. FT-IR spectra of (a) AgNPs, and (b) Li-AgNPs

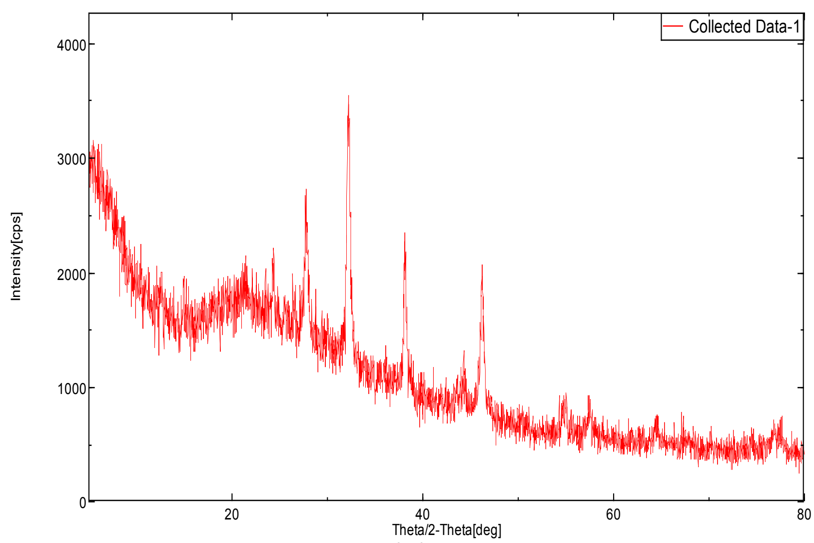
can be assigned to O-H stretch, N-H, C-H, O-H, and C=C groups, respectively (Fig.3b). The absorption band at 2065 cm^{-1} confirms adsorption of antibiotic linezolid on AgNPs.

XRD analysis

The synthesized AgNPs and Li-AgNPs were subjected to XRD analysis for the measurement of sizes of these particles. The XRD pattern obtained for the AgNPs shows 2 theta values of 20.57^0, 22.95^0, 32.40^0, 44.27^0, 64.66, and 77.63^0 appeared, indexed as crystalline silver (Fig. 4a). The XRD pattern of Li-AgNPs showed 2 theta values of 27.81^0, 32.22^0, 38.14^0, 44.34^0, 46.26^0, and 76.94^0 (Fig. 4b). The sharp peaks clearly indicate that the nanoparticles are spherical in shape and crystalline in nature.



(a)



(b)

Fig 4. XRD analysis of (a) AgNPs, and (b) Li-AgNPs

Antimicrobial activity of AgNPs and Li- AgNPs

The antimicrobial potency of *T. populnea* leaf extract, AgNPs, Linezolid conjugated AgNPs and Linezolid was assessed against *E. faecium, E. faecalis*, and *S. aureus*. The results showed that a significant microbial growth inhibition was observed with both AgNPs as well as Li-AgNPs (Fig. 5&6). The synthesized Li-AgNPs have

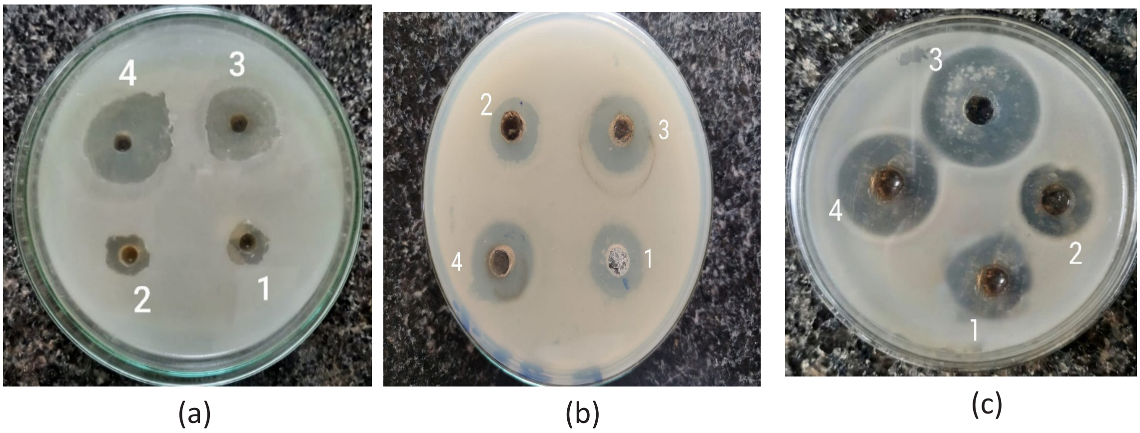


Fig 5. Petri plates showing antimicrobial activity of (1) T. *populnea* leaf extract, (2) AgNPs, (3) Li-AgNPs, and (4) Linezolid against (a) *E. faecium*, (b*) E. faecalis*, and (c) *S. aureus*

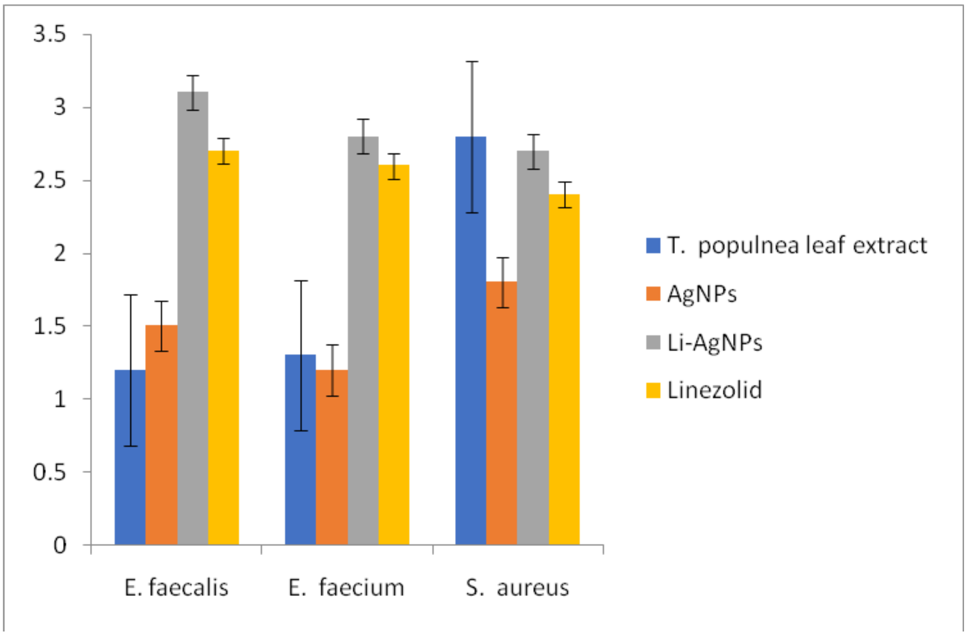


Fig 6. Bar graph showing antimicrobial activity of T. *populnea* leaf extract, AgNPs, Li-AgNPs and Linezolid against *E. faecalis, E. faecium*, and *S. aureus*

Table1. Inhibition zones of (1) T. *populnea* leaf extract, (2) AgNPs, (3) Li-AgNPs, and (4) Linezolid against *E. faecalis, E. faecium,* and *S. aureus*

Name of Microorganism	Zone of Inhibition (cm)#			
	T. populnea leaf extract	AgNPs	Li-AgNPs	Linezolid
E. faecalis	1.2±0.1	1.5±0.2	3.1±0.2**	2.7±0.2*
E. faecium	1.3±0.1	1.2±0.1	2.8±0.3**	2.6±0.2
S. aureus	1.8±0.1	1.8±0.2	2.7±0.1**	2.4±0.1

#Values represent the mean (± SE) from three experiments, (n = 4)
*The values of Li-AgNPs were significantly compared with Linezolid ($p < 0.005$)
**The values Li-AgNPs were significantly compared with the AgNPs ($p < 0.002$)

shown 25.8%, 7.6%, and 12.5% more growth inhibition activity against *E. faecalis*, *E. faecium*, *and S. aureus*, respectively, compared to standard antibiotic Linezolid (Table 1).

CONCLUSION

Nowadays, MDR bacteria are posing a greater trouble by showing resistance against commonly used antibiotics. Hence, scientists have a great task to find a novel strategy to enhance the efficiency of antibiotics. In the present study, we have demonstrated the use of a natural, low-cost, biological reducing and capping agent i.e., *T. populnea* leaf extract to produce metal(Ag) nanoclusters through green methodology. The formation of AgNPs was confirmed by UV-visible spectroscopy. The shape, crystallinity, and functional groups present in the synthesized AgNPs were studied by SEM, XRD, and FT-IR spectra through the distinct characteristic peaks. The antimicrobial activity of synthesized AgNPs and Li-AgNPs were evaluated against three MDR bacterial pathogens such as *S. aureus*,*E. faecalis*, and E. *faecium* by standard well diffusion method. The data clearly indicates that the antibiotic linezolid conjugation with AgNPs greatly enhanced the antimicrobial activity in the decreasing order from *E. faecalis*,*E. faecium*,and *S. aureus*,respectively. The Li-AgNPs also showed potent inhibitory effect than *T. populnea* plant extract, AgNPs, and antibiotic linezolid. These results clearly demonstrate the utility of Li-AgNPs in enhancing the antibacterial potency of both AgNPs as well as Li-AgNPs. The present study showed a simple, rapid, and economical route to synthesize AgNPs and Li-AgNPs that could be employed for clinical trials thereby enhancing the antibacterial effect of currently used antibiotics to cure various communicable diseases caused by MDR pathogens. The use of T. *populnea* has an added advantage that this plant can be used as antibacterial and in wound healing. This method is potentially exciting for the large-scale synthesis of nanoparticles.

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ETHICAL APPROVAL

Animals are not used in the experiments, so no need of ethical approval.

DISCLOSURE STATEMENT

The authors report there are no competing interests to declare.

FUNDING

None

REFERENCES

1. Raghavendra Shivamogga Nagaraju, Raghu Holalkere Sriram, Rajeshwara Achur.Antifungal activity of Carbendazim-conjugated silver nanoparticles against anthracnose disease caused by *Colletrichumgloeosporiodes* in mango.Plant Pathol J.2020;102: 39-46.
2. Raghavendra S.N, Raghu H.S, Divyashree K, Rajeshwara A.N. Antifungal Efficiency Of Copper Oxychloride-conjugated Silver Nanoparticles Against Colletotrichum Gloeosporioides which causes Anthracnose Disease.Asian J Pharm Clin Res. 2019;12(8): 230-233.

3. RaghavendraS.N, RaghuH.S, Chithra C, Rajeshwara A.N. Potency of Mancozeb Conjugated Silver Nanoparticles Synthesised from Goat, Cow and Buffalo Urine sample against Colletotrichum gloeosporiodies Causing Anthracnose Disease.Nat Environ Pollut Technol. 2020; 19(3).
4. Raghu H.S, Raghavendra S.N, Malini B.P, Chaitra C, Ananth NagB.Synthesis of silver nanoparticles from goat urine (Ajamuthra) and their antifungal efficacy against *Colletotrichum gloeosporioides.* Pollut Res.2021; 40(3): 918-921.
5. MalhotraR. Mass spectroscopy in life sciences. Curr Sci. 2010; (98):140–145,
6. PrabhuN, RajD.T, Yamuna GowriK, Aiysha Siddique S, Joseph Pushpa Innocent D.Synthesis of silver Phyto nanoparticles and their antibacterial efficacy.Digest J Nanomater Biostruct. 2010;5(1): 185–189.
7. Balaji S. Nanobiotechnology. MJP Publisher; 2019
8. NairB,Pradeep T.Coalescence of nanoclusters and the formation of sub-micron crystallites assisted by Lactobacillus strains. Cryst Growth Des. 2002;4 (2): 293–298.
9. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, Sastry M. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano Lett. 2001;10 (1):515–519
10. Duran N, MarcatoP. D, AlvesO. L, De SouzaG. I. H, E.´Esposito. Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium auxospore strains. J Nanobiotechnology. 2005;3:1-7.
11. ChandranS P, ChaudharyM, PasrichaR, Ahmad A, Sastry M.Synthesis of gold nanotriangles and silver nanoparticles using Aloe Vera plant extract. Biotechnol Prog. 2006;22 (2):577–583.
12. LiS, ShenY, Xia A,et al.Green synthesis of silver nanoparticles using Capsicum annuum L. extract. Green Chem. 2007; 9(8):852–858.
13. HuangJ, LiQ, Sun D. et al.Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphor leaf. Nanotechnol. 2007;18(10):105104.
14. Garima S, RajuB, KunalK, AshishR S, RajendraP S.Biosynthesis of silver nanoparticles using Osmium sanctum (Tullis) leaf extract and screening its antimicrobial activity.J Nanoparticle Res. 2011;13(7): 2981–2988.
15. SinghA, JainD,UpadhyayM K, KhandelwalN, VermaH N. Green synthesis of silver nanoparticles using Argemone Mexicana leaf extract and evaluation of their antimicrobial activities. Digest J Nanomater Biostruct. 2010; 5(2):483–489.
16. ParikhD V, FinkT,Rajasekhara K,et al.Antimicrobial silver/sodium carboxymethyl cotton dressings for burn wounds. Text Res J. 2005; 75(2): 134–138.
17. AltV, BechetT, SteinruckeP. et al.An invitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. Biomater. 2004; 25(18): 4383–4391.
18. Gosh EgerG,HardeesJ,Ahrens H. et al.Silver-coated mega endoprostheses in a rabbit model—an analysis of the infection rate and toxicological side effects.Biomater. 2004; 25(24):5547–5556.
19. RuppM E,FitzgeraldT,Marion N,et al.Effect of silver coated urinary catheters: efficacy, cost-effectiveness, and antimicrobial resistance. Am J Infect Control. 2004;32(8): 445–450.
20. OhashiS, SaukS,Yamamoto K.Antibacterial activity of silver inorganic agent YDA filler. J Oral Rehabil. 2004;31(4):364–367.
21. BosettiM, MasseA, TobinE, Cannas M.Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity.Biomater.2002; 23(3): 887–892.
22. Lee H Jand JeorgS H. Bacteriostasis and skin innoxiousness of nanosized silver colloids on textile fabrics. Text Res J.2005;75(7):551–556.
23. SarkarR, KumbhakarP, MitraA K. Green synthesis of silver nanoparticles and its optical properties. Digest J Nanomater Biostruct.2010;5(2): 491–496.
24. Mohan raj V J, Chen Y. Nanoparticles-A Review. Trop J Pharm.2006;5(1):561-573.
25. Eisen GA. The Fig: Its History, Culture, and Curing: With a Descriptive Catalogue of the Known Varieties of Figs. US Government Printing Office; 1901.
26. RHS A-Z encyclopaedia of garden plants. United Kingdom: Dorling Kindersley.2008; 1136.
27. FarooquiM D A, ChauhanP S, KrishnamoorthyP, Shaik J. Extraction of silver nanoparticles from the left extracts of cleroterium incerme. Digest J Nanomater Biostruct.2010;5(1):43–49.
28. IpM,LuS L, PoonV K M, LungI, Bard A.Antimicrobial activities of silver dressings: an in vitro comparison. J Med Microbiol.2006;55(1):59–63.
29. ShankarS S, RaiA, AhmadA, Sastry M. Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings. Mater Chem.2005; 17(3):566– 572.
30. WileyB J, ImS H, LiZ-Y, McLellanJ, SiekkinenA, Xia Y.Manoeuvring the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. J Phys Chem B.2006;110(32):15666–

15675.
31. Friday JB, Okano D. Thespesia populnea (milo). Species profiles for Pacific island agroforestry. 2006 Apr:19.
32. Prema P. Chemical mediated synthesis of silver nanoparticles and its potential antibacterial application. Progress in molecular and environmental bioengineering-from analysis and modeling to technology applications. 2011 Aug 1;6:151-66.
33. Wang ZL. Transmission electron microscopy and spectroscopy of nanoparticles. Characterization of nanophase materials. 1999:37-80.
34. SavithrammaN,Linga RaoM, Suvarnalatha Devi P. Evaluation of antibacterial efficacy of biologically synthesized silver Nanoparticles using stem barks of Boswellia ovalifoliolata Bal. and Henry and Shoreatumbuggaia Roxby. J Biol Sci.2011;11(1):39–45.
35. BauerA W, KirbyW W M,SherrisJ C, Truck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol.1966;45(4):493–496.
36. SastryM,AhmadA,KhanM I, KumarR. Biosynthesis of metal nanoparticles using fungi and actinomycetes. Curr Sci.2003;85(2): 202–206.
37. Mohapatra Pai R K. Engineering Chemistry with laboratory experiments, 1st edition, PHI learning, Delhi, 30th september 2015.
38. PalS, TekY K,SongJ M.Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Appl Environ Microbiol. 2007;73(6): 1712– 1720.