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Green Synthesis and Characterization of Silver Nanoparticles from *Ficus palmata* Forssk. and Evaluation of their Antibacterial Activity Against Resistant Bacteria

Tayyab Rehman[1], Hoor Shumail[2*], Shah Khalid[3*], Syed Inzimam Ul Haq[3]
[1]*Faculty of Allied Health Sciences, Iqra National University, Swat, Khyber Pakhtunkhwa, 19220, Pakistan.*
[2]*Department of Microbiology, Women University Mardan, Khyber Pakhtunkhwa, 23200, Pakistan.*
[3]*Department of Botany, Islamia College Peshawar, Khyber Pakhtunkhwa, 25120, Pakistan.*

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ABSTRACT

In the present study, the synthesis and characterization of silver nanoparticles were carried out through a green chemistry approach. The purpose was to determine the antibacterial potential of environmentally safe nanoparticles against commonly reported resistant bacteria. The nanoparticles were synthesized using the aqueous extract of *Ficus palmata* Forssk. leaves. The active components present in the leaf extract acted as reducing and capping agents. The method used was easy, cost-effective, eco-friendly, and less time-consuming. To determine the shape, size, and crystalline nature, the nanoparticles underwent X-ray diffraction, and scanning electron microscopy. The synthesized nanoparticles were found to be crystalline in nature and spherical in shape, with an average diameter of about 30 nm. The antibacterial activity of the silver nanoparticles (20 µl) was evaluated against a range of antibiotic-resistant bacteria using standard microbiological procedures. They were found to be effective against MRSA, *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumonia*.

Corresponding Author: Hoor Shumail; Shah Khalid
Email: hoorshumail12@gmail.com; shahkhalid@icp.edu.pk

INTRODUCTION

Antibiotic resistance is a rapidly growing threat to the world. As a result, it is becoming more challenging to treat many infectious diseases (Murray *et al*., 2022). Over time, microorganisms are developing resistance to the available antimicrobials, highlighting the urgent need to develop alternatives for treating these resilient microorganisms (Rudramurthy *et al*., 2016; Frieri *et al*., 2017; Kongkham *et al*., 2020; Larsson and Flach, 2021). Target-specific resistance has been developed against nearly every class of antibiotic (Schmieder and Edwards, 2012; Tiwari *et al*., 2013; Hassoun-Kheir *et al*., 2020; Stracy *et al*., 2022; He *et al*., 2022; Zhu *et al*., 2022). Due to drug resistance, antibiotics are frequently administered in high doses, leading to potential toxicity. This scenario has triggered the need to explore alternative and innovative approaches to manage bacterial infections. Among the challenging clinical infections that require special attention are those caused by the ESKAPE pathogens, namely *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacter species. ESKAPE species are known for their opportunistic nature and are commonly associated with nosocomial infections (Ma *et al*., 2020; Das *et al*., 2022). These bacteria have developed a significant level of antibiotic resistance through multiple mechanisms. The acronym ESKAPE serves as a symbolic representation of the ability of these bacteria to "escape"

eradication by antibiotics and highlights the stubborn nature of infections caused by ESKAPE pathogens, which often show resistance to conventional therapies (Pendleton *et al*., 2013; Mulani *et al*., 2019; Ma *et al*., 2020; De Oliveira *et al*., 2020; Nasser *et al*., 2020).

Nanoscale materials have emerged as innovative antimicrobial agents, providing an alternative approach to combat pathogens. Various nanoparticles and nanosized carriers have been demonstrated to be effective in treating bacterial infections and facilitating drug delivery, as evidenced by studies conducted in vitro and in animal models (Ravishankar and Jamuna Bai, 2011; Hepokur *et al*., 2019; Fatima *et al*., 2021). Among metal nanoparticles, silver nanoparticles have garnered significant attention due to their excellent antimicrobial properties. Compared to traditional antibacterial agents, silver nanoparticles exhibit enhanced disinfectant and antibacterial characteristics. This is primarily attributed to their high surface area to volume ratio, which gives rise to distinct chemical, electrical, optical, magnetic, and mechanical properties that differ from those of the bulk material of the same type. Notably, these properties are largely influenced by the particle size of silver nanoparticles (Hajipour *et al*., 2012; Le Ouay and Stellacci, 2015; Hamad *et al*., 2020; Pandit *et al*., 2022). Silver ions have been found to inhibit bacterial DNA replication, induce damage to cytoplasmic membranes, and reduce ATP levels. These actions ultimately lead to cell death in bacteria (Franci *et al*., 2015; Bruna *et al*., 2021). This suggests that the nanoparticles exhibit effectiveness against both gram-positive and gram-negative bacteria.

A new and alternative approach to treating antibiotic-resistant pathogens involves the utilization of medicinal plants. These plants serve as valuable sources of various chemical compounds such as phenols, flavonoids, glycosides, alkaloids, saponins, triterpenes, and more. These chemical constituents can be potentially harnessed for the synthesis of new drugs with therapeutic properties (Savoia, 2012; Mahboubi, 2021). Using natural plant products as drugs offers several advantages, including safety, cost-effectiveness, and easy availability, with minimal side effects (Chanda, 2013; Rajput, 2015; Osungunna, 2021). Plants are often considered a favorable option for the treatment of various infectious diseases, and their extracts are commonly utilized for this purpose. In addition to plant extracts, nanoparticles have been developed using plant materials and employed against various microbes (Osungunna, 2021; Mishra *et al*., 2014; Ugboko *et al*., 2020). Nanoparticles can be synthesized through various mechanisms. Among them, the green chemistry approach is considered the most cost-effective, environmentally friendly, and non-hazardous method for nanoparticle synthesis (Rout *et al*., 2012; Jadoun *et al*., 2021; Shumail *et al*., 2021). Plants contain a diverse range of active components that serve as reducing, capping, and stabilizing agents during nanoparticle synthesis. The biomolecules present in plants, including phenols, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids, and alcoholic compounds, play a significant role in controlling the shape and stability of nanoparticles (Makarov *et al*., 2014; Behera *et al*., 2020; Shumail *et al*., 2021). Phenolic compounds possess carboxyl and hydroxyl groups, which facilitate their binding to metals during the synthesis process. These functional groups allow for effective interaction and coordination with metal ions, contributing to the synthesis of metal nanoparticles (Rauwel *et al*., 2015). *Ficus palmata* belongs to the family Moraceae (Harrison, 2005; Sbhatu *et al*., 2020). Several species of Ficus are utilized in traditional medicine for their anti-inflammatory, anti-tumor, and tonic properties. These species have a long history of being used as medicinal remedies (Kitajima *et al*., 1999; Ogunlaja *et al*., 2022; Khan *et al*., 2022). Ficus extracts have been reported to have potential benefits in treating a variety of diseases, including epilepsy, jaundice, influenza, whooping cough, tonsillitis, bronchitis, enteritis, bacillary dysentery, toothache, and bruises (Kitajima *et al*., 1999).

The objective of the present study was to assess the capability of fresh leaves of *Ficus palmata* in synthesizing silver nanoparticles (AgNPs). Furthermore, the study aimed to investigate the antimicrobial properties of these nanoparticles against both gram-positive and gram-negative microorganisms.

MATERIAL AND METHODS

Sample Collection and species identification

Fresh leaves of Ficus were collected from the University Campus in Peshawar and subsequently sent to the Centre of Plant Biodiversity at the University of Peshawar for species identification.

Preparation of plant extract and silver nitrate solution

Fresh leaves of *Ficus palmata* were carefully washed with distilled water to eliminate any dust particles. Following

the washing process, the leaves were air dried for approximately 25-30 minutes and then finely chopped into small pieces. These chopped leaves were subsequently boiled in distilled water for 5 minutes. The resulting extract was separated from the leaf pieces through decanting, and this solution was utilized for the reduction of silver ions to silver nanoparticles. Concurrently, a 1mM solution of silver nitrate (Sigma Aldrich) was prepared by dissolving 0.0076 grams of silver nitrate in 45 ml of distilled water.

Synthesis of silver nanoparticles (AgNPs)

The prepared silver nitrate solution was placed in a water bath set at 55°C. Then, 5 ml of the prepared leaf extract was added drop by drop into the silver nitrate solution while continuously stirring at 55°C. The color of the silver nitrate solution changed from light green to light brown, indicating the formation of silver nanoparticles (Figure 1).

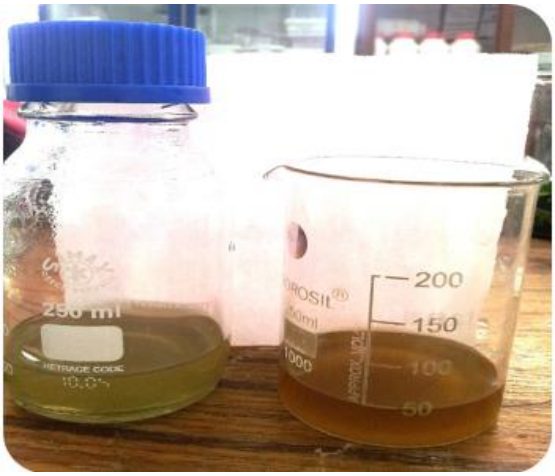

Figure 1. Synthesis of Silver Nanoparticles.

Characterization of nanoparticles

X-Ray Diffraction: X-Ray diffraction (XRD) was employed to obtain structural information regarding the crystalline compounds by analyzing the diffraction pattern produced by dual waves of X-rays. In this study, X-ray diffraction technique (JDX 3532, Jeol Japan) was utilized for phase identification of the samples, employing Kα radiation. The generator voltage was set at 40 Kv, and the current was maintained at 30mA. The samples were scanned within the 2θ range. The crystalline size of the nanoparticles was determined using the Scherrer equation: $Dp = 0.94\lambda/\beta\cos\theta$.

SEM: The morphology and size of the nanoparticles were investigated using a scanning electron microscope (Jeol, JSM 5910). A small drop of the sample was placed on a glass slide coated on both sides with conducting carbon tape. The surface of the sample was then coated with a layer of gold using a gold sputter coater (SPI Company, USA) at 30 Dc mA for 1.5 minutes. The magnification range used for imaging was 2,000-60,000x, and the acceleration voltage was set at 20Kv.

Antibacterial activity

Samples collection: Antibiotic-resistant bacterial strains, including *Enterococcus faecium*, *Escherichia coli*, MRSA (Methicillin-Resistant Staphylococcus aureus), *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella paratyphi* A, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Acinetobacter baumannii*, were collected from tertiary care hospitals in Peshawar. These strains were stored and maintained at 4ºC for further analysis and experimentation.

Assessing antibacterial activity: For the antibacterial activity assessment, an inoculum was prepared using the direct colony suspension technique and compared with a 0.5 McFarland standard. The turbidity of the inoculum was adjusted accordingly. A sterile swab was immersed in the inoculum and rotated against the walls of the test tube to remove excess liquid. To ensure uniform distribution of the inoculum, the entire surface of a Mueller-Hinton Agar (MHA) plate was swabbed three times by rotating the plate at an angle of 60 degrees. Wells were then created on the plates to accommodate 20µl of silver nanoparticles and silver nitrate solution. Antibiotic disks were placed on the MHA surface with gentle pressure to ensure complete contact with the agar. The plates were allowed to stand in a laminar flow hood (LFH) for 30 minutes to allow the nanoparticles to diffuse into the agar media. After 30 minutes, the plates were placed

in an incubator at a temperature of 35±2°C for 16-18 hours for bacterial growth.

Zone of inhibition formation: After 17 hours of incubation, zones of inhibition were observed around the positive control for all bacterial strains, indicating the effectiveness of the chosen antibiotics against those strains. No zones of inhibition were observed for the negative control for any of the bacterial strains. Regarding the silver nanoparticles, zones of inhibition were observed for only four out of the ten tested bacterial strains. Among these four strains, three were Gram-positive and one was Gram-negative. The zones of inhibition were carefully observed and measured to assess the antimicrobial activity of the silver nanoparticles against the respective strains.

RESULTS

Activity of silver nanoparticles against Gram-negative bacteria

The prepared nanoparticles solution was evaluated against five Gram-negative bacteria strains, namely *E. coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* A, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. The results indicated that the nanoparticles exhibited effectiveness against only one of the Gram-negative bacteria, specifically *Klebsiella pneumoniae*. The zone of inhibition formed around the *Klebsiella pneumoniae* colony had a diameter of 32mm, while the positive control, imipenem, formed a zone with a diameter of 20mm. These measurements were compared to the guidelines provided by the Clinical Laboratory Standards Institute (CLSI) in 2015. The zone of inhibition for impenem with a diameter of 20mm is considered to be intermediate resistant. It is evident from table 1, that the nanoparticles demonstrated significantly higher effectiveness against *Klebsiella pneumoniae* compared to the antibiotic imipenem. The observed zone of inhibition for the nanoparticles was much larger (32mm) in comparison to the zone of inhibition formed by imipenem. This suggests that the nanoparticles have a stronger antimicrobial effect against *Klebsiella pneumoniae* than the imipenem antibiotic.

According to the CLSI 2015 guidelines, *Acinetobacter baumannii* displayed resistance to imipenem with a zone of inhibition of 6mm, which is below the threshold of 19mm for susceptibility. *E. coli*, *Pseudomonas aeruginosa*, and *Salmonella paratyphi* A showed intermediate resistance to imipenem with a zone of inhibition of 20mm, which falls within the intermediate range according to CLSI guidelines. Regarding the nanoparticles, it is noted that all gram-negative isolates, except for *Klebsiella pneumoniae*, exhibited resistance as there was no observed zone of inhibition. These results highlight the varying susceptibility patterns of different bacterial strains to both imipenem and the tested nanoparticles. *Klebsiella pneumoniae* displayed susceptibility to both the nanoparticles and imipenem, while the other gram-negative strains showed resistance or intermediate resistance to imipenem and resistance to the nanoparticles (Table 1).

Table 1. Summary of NP's activity against Gram-negative resistant bacteria in comparison with positive and negative controls.

Sr. No	Test Organisms	Zone of inhibition diameter (mm)														
		NP's							Positive Control (Imipenem disc)							Negative Control ($AgNO_3$ solution)
		Strains						Avg.	Strains						Avg.	
		1	2	3	4	5	6		1	2	3	4	5	6		
1	*Escherichia coli*	8	0	9	11	8	0	6	20	7	25	28	22	18	20	0
2	*Pseudomonas aeruginosa*	--	--	0	--	9	0	3	--	--	20	--	18	22	20	0
3	*Klebsiella pneumonia*	31	32	--	33	--	--	32	19	20	--	21	--	--	20	0
4	*Salmonella paratyphi A*	0	11	9	10	--	0	6	19	21	19	21	--	20	20	0
5	*Acinetobacter baumannii*	--	0	--	--	0	--	0	--	0	--	--	6	--	6	0

Activity of silver nanoparticles against Gram-positive bacteria

S The prepared solution of nanoparticles was tested against five strains of gram-positive bacteria, i.e., *Enterococcus faecium*, *Staphylococcus aureus*, MRSA, *Bacillus subtilis*, and *Streptococcus pyogenes*. According to the results, the nanoparticles were effective against three gram-positive bacteria, namely MRSA, *Staphylococcus aureus*, and *Bacillus subtilis*, showing a zone of inhibition with diameters of 32 mm, 28 mm, and 24 mm, respectively. According to CLSI 2015 guidelines, MRSA is found to have a zone of 30 mm against imipenem, and a zone above 23 mm is considered susceptible. There is not much difference between the zone of imipenem (30 mm) and the zone of nanoparticles (32 mm), which suggests that MRSA is almost equally susceptible to imipenem and nanoparticles. *Staphylococcus aureus* is found to have a zone of 24 mm, which falls within the range of susceptibility. However, the nanoparticles have a zone of 28 mm, indicating that nanoparticles have a greater effect compared to the antibiotic used. For *Bacillus subtilis*, imipenem has a zone of 20 mm, which is considered to be intermediate resistance. On the other hand, nanoparticles have a zone of 24 mm, suggesting that nanoparticles are more effective than imipenem. *Enterococcus faecium* and *Streptococcus pyogenes* have produced zones of 20 mm and 21 mm, respectively, indicating that these isolates are intermediate resistant to imipenem according to CLSI 2015 guidelines, and they are completely resistant to nanoparticles (Table 2).

Table 2. Summary of NP's activity against Gram-positive resistant bacteria in comparison with positive and negative controls.

Sr. No	Test Organisms	Zone of inhibition diameter (mm)														
		NP's							Positive Control (Imipenem disc)							Negative Control ($AgNO_3$ solution)
		Strains						Avg.	Strains						Avg.	
		1	2	3	4	5	6		1	2	3	4	5	6		
1	*Enterococcus feacium*	0	0	8	0	--	--	2	20	19	22	19	--	--	20	0
2	*Staphylococcus aureus*	28	27	29	29	28	27	28	24	24	25	24	24	23	24	0
3	MRSA	31	32	32	33	31	33	32	29	30	30	31	29	31	30	0
4	*Bacillus subtilis*	25	--	24	23	24	--	24	21	--	20	19	20	--	20	0
5	*Streptococcus pyogenes*	--	0	--	0	0	--	0	--	21	--	20	22	--	21	0

Characterization of nanoparticles

X-Ray diffraction

The sharp peaks indicate the crystalline nature of the nanoparticles. The XRD pattern shows diffraction lines at low angles, ranging from 10^0 to 75^0. The observed diffraction lines have 2θ angles of 40.0^0, 44.7^0, and 65.9^0. The XRD patterns were evaluated to determine peak intensity, width, and full width at half maximum data. These data were then used to calculate the particle size using the Scherer equation (Figure 2). The average particle size was found to be approximately 30 nm, confirming their crystalline nature.

Scanning Electron Microscopy

The surface morphology of the nanoparticles was studied using a scanning electron microscope (SEM). The micrographs obtained from the SEM analysis provide a clear image of nanoparticles that are approximately spherical in shape (Figure 3).

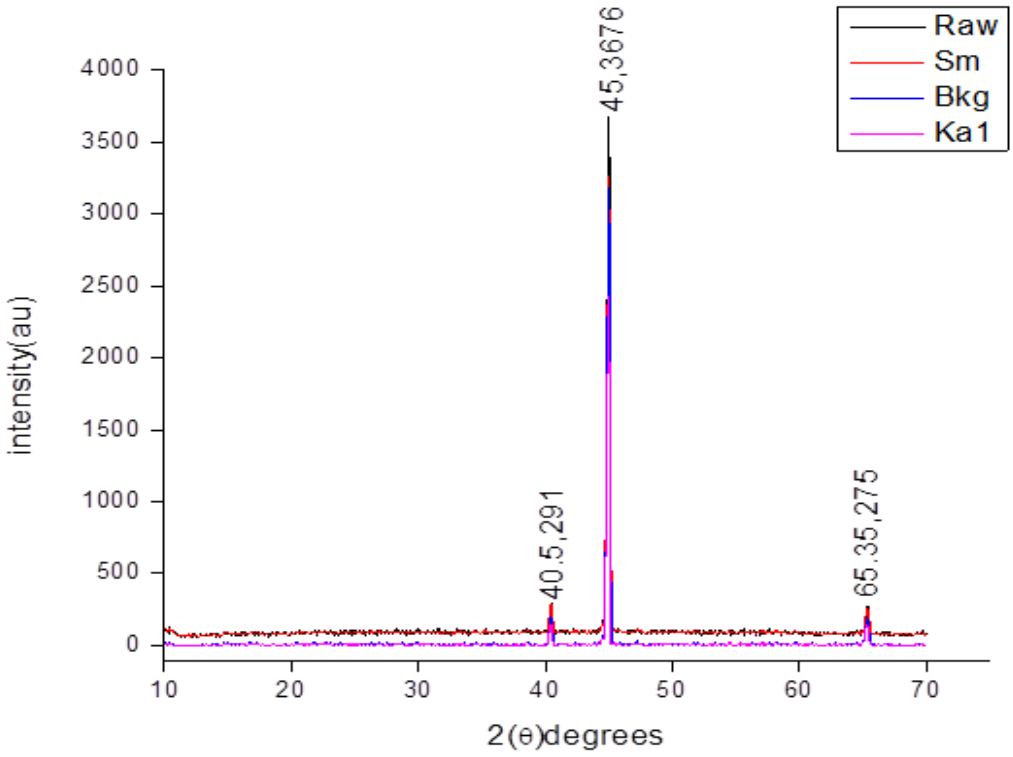

Figure 2. XRD pattern showing peaks at 40.0^0, 44.7^0 and 65.9^0.

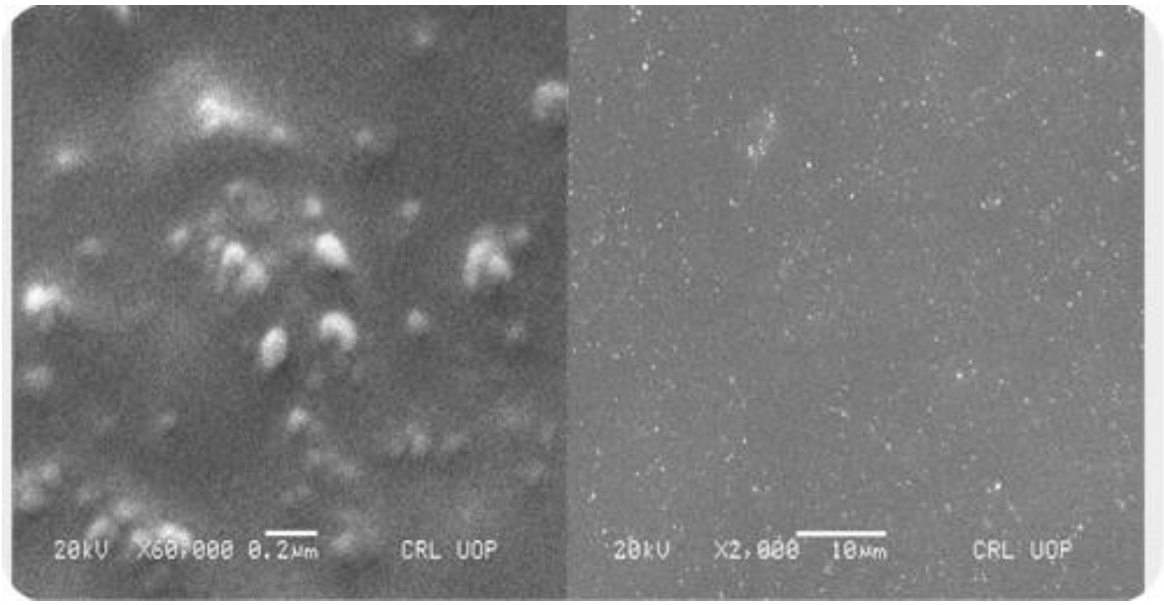

Figure 3. SEM images showing the shape of nanoparticles.

All the above results suggest that the nanoparticles were successfully synthesized using leaf extract, which confirms that *Ficus palmata* contains important components that act as reducing agents, completing the process of nanoparticle synthesis.

DISCUSSIONS

With the passage of time, nanotechnology is gaining increasing importance due to its potential applications in various fields. The nanoparticles synthesized using plant extract have been found to be more stable compared to nanoparticles synthesized using other organisms (Singh *et al*., 2016). In addition to their stability, nanoparticles synthesized using plant extracts offer the advantage of time efficiency, as most plants are available throughout the year. Unlike other organisms, such as microbes, plants retain their potential for nanoparticle synthesis even when kept in culture for extended periods (Ahmed *et al*., 2016). The nanoparticles synthesized using plant extract have significant potential applications in various fields, including pharmaceuticals, food, medicine, diagnostics, cancer treatment, and drug delivery. They are considered safe, biocompatible, and non-toxic. Currently, pilot studies are being conducted to assess and evaluate their potential for human benefits. The antibacterial activity of silver nanoparticles primarily depends on the concentration of silver nitrate. Nanoparticles with lower metal concentration are considered to be more effective than those with higher metal concentration. It has been reported that silver ions cause similar morphological alterations in both gram-negative and gram-positive bacteria by detaching the cell membrane from the cytoplasmic membrane (Sahayaraj and Rajesh, 2011).

In the present study, silver nanoparticles synthesized using aqueous leaf extract of *Ficus palmata* were employed to investigate their antibacterial potential against commonly resistant bacteria. The antimicrobial potential was determined using the well diffusion assay, which has also been previously reported in similar studies (Gavhane *et al*., 2012; Murugan *et al*., 2014; Kamath and Packiyam, 2017). In previous studies, the use of silver nitrate as a negative control was reported. It was observed that the silver nitrate solution showed no zone of inhibition in response to bacterial growth (Gavhane *et al*., 2012).

Our synthesized silver nanoparticles were found to exhibit activity against *Staphylococcus aureus*, which is consistent with previous studies conducted on similar nanoparticles (Sarsar *et al*., 2013; Khalil *et al*., 2014). In this study, the nanoparticles demonstrated limited activity against MRSA compared to the antibiotic used. This finding aligns with the work done by Arunkumar *et al*., (2014). However, it is worth noting that the previous study utilized the disc diffusion method, which may account for the difference in results (Arunkumar *et al*., 2014). The synthesized nanoparticles were found to be effective against *Bacillus subtilis*, which is consistent with previous studies conducted on similar nanoparticles (Gnanajobitha *et al*., 2013; Awwad *et al*., 2013). The nanoparticles were found to be ineffective against all four gram-negative pathogens used in the research work. However, the results obtained for *E. coli* differ significantly from the findings of previous studies (Khalil *et al*., 2014; Roy *et al*., 2015). Additionally, the nanoparticles were found to be ineffective against *Pseudomonas aeruginosa*, which also deviates from the findings of previous studies (Savithramma *et al*., 2011; Lalitha *et al*., 2013).

CONCLUSION

In this study, silver nanoparticles were successfully synthesized using the aqueous extract of *Ficus palmata* leaves. The active components within the leaf extract played crucial roles as reducing, capping, and stabilizing agents in the synthesis process. The chosen method offered several advantages such as simplicity, speed, cost-effectiveness, and excellent performance. Confirmation of nanoparticle synthesis was achieved through the observation of a color change in the silver nitrate solution, transitioning from transparent to light brown, indicating successful nanoparticle formation. Additional confirmation was obtained through analytical techniques such as X-ray diffraction (XRD) and scanning electron microscopy (SEM), which revealed the crystalline nature of the nanoparticles and their spherical shape, with an average diameter of approximately 30 nm. The synthesized silver nanoparticles were then subjected to antimicrobial testing against a range of antibiotic-resistant bacterial isolates, including *Enterococcus faecium*, *E. coli*, MRSA, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella paratyphi* A, *Streptococcus pyogenes*, *Acinetobacter baumannii*, and *Staphylococcus aureus*. The results showed that the nanoparticles exhibited a zone of inhibition against three gram-positive bacterial isolates and one gram-negative bacterial isolate. These zones of inhibition were

compared to the zone of Imipenem, a Carbapenem class antibiotic considered a last resort for treating resistant bacteria. Notably, the synthesized nanoparticles demonstrated significant activity against certain pathogenic isolates, highlighting their potential as a promising alternative to traditional antibiotics. Overall, this study successfully synthesized silver nanoparticles using *Ficus palmata* leaf extract and demonstrated their antimicrobial efficacy against specific antibiotic-resistant bacterial strains. These findings support the future exploration of silver nanoparticles as a viable option for combating microbial infections and potentially overcoming the challenges posed by antibiotic resistance. Further research and development in this area are warranted to fully evaluate the efficacy, safety, and potential applications of silver nanoparticles in various fields, including medicine and healthcare.

AUTHORS CONTRIBUTION STATEMENT

Shah Khalid and Hoor Shumail designed the experiment; Tayyab Rehman performed the experiments; Syed Inzimam Ul Haq wrote the manuscript; Shah Khalid revised the manuscript; Tayyab Rehman commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Ahmed, S., M. Ahmad, B.L. Swami and S. Ikram. 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of Advanced Research, 7(1): 17-28.

Arunkumar, M., K. Suhashini, N. Mahesh and R. Ravikumar. 2014. Quorum quenching and antibacterial activity of silver nanoparticles synthesized from *Sargassum polyphyllum*. Bangladesh Journal of Pharmacology, 9(1): 54-59.

Behera, A., B. Mittu, S. Padhi, N. Patra and J.J. Singh. 2020. Bimetallic nanoparticles: Green synthesis, applications, and future perspectives. In Multifunctional hybrid nanomaterials for sustainable agri-food and ecosystems. Elsevier, 639-682.

Bruna, T., F. Maldonado-Bravo, P. Jara and N. Caro. 2021. Silver nanoparticles and their antibacterial applications. International Journal of Molecular Sciences, 22(13): 7202.

Chanda, S. 2013. Silver nanoparticles (medicinal plants mediated): A new generation of antimicrobials to combat microbial pathogens-a review. Microbial pathogens and strategies for combating them: Science, Technology and Education, 1314-1323.

CLSI. M100-S25 performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement; 2015.

Das, S., S. Bombaywala, S. Srivastava, A. Kapley, R. Dhodapkar and N.A. Dafale. 2022. Genome plasticity as a paradigm of antibiotic resistance spread in eskape pathogens. Environmental Science and Pollution Research, 29: 40517-40529.

De Oliveira, D.M., B.M. Forde, T.J. Kidd, P.N. Harris, M.A. Schembri, S.A. Beatson, D.L. Paterson and M.J. Walker. 2020. Antimicrobial resistance in ESKAPE pathogens. Clinical Microbiology Reviews, 33(3): e00181-19.

Fatima, F., S. Siddiqui and W.A. Khan. 2021. Nanoparticles as novel emerging therapeutic antibacterial agents in the antibiotics resistant era. Biological Trace Element Research, 199(7): 2552-2564.

Franci, G., A. Falanga, S. Galdiero, L. Palomba, M. Rai, G. Morelli and M. Galdiero. 2015. Silver nanoparticles as potential antibacterial agents. Molecules, 20(5): 8856-8874.

Frieri, M., K. Kumar and A. Boutin. 2017. Antibiotic resistance. Journal of infection and public health, 10(4): 369-378.

Gavhane, A.J., P. Padmanabhan, S.P. Kamble and S.N. Jangle. 2012. Synthesis of silver nanoparticles using extract of neem leaf and triphala and evaluation of their antimicrobial activities. International Journal of Pharma and Bio Sciences, 3(3): 88-100.

Gnanajobitha, G., K. Paulkumar, M. Vanaja, S. Rajeshkumar, C. Malarkodi, G. Annadurai and Kannan. 2013. Fruit-mediated synthesis of silver nanoparticles using *Vitis vinifera* and evaluation of their antimicrobial efficacy. Journal of Nanostructure in Chemistry, 3: 1-6.

Hajipour, M.J., K.M. Fromm, A.A. Ashkarran, D.J. de

Aberasturi, I.R. de Larramendi, T. Rojo, V. Serpooshan, W.J. Parak and M. Mahmoudi. 2012. Antibacterial properties of nanoparticles. Trends in Biotechnology, 30(10): 499-511.

Hamad, A., K.S. Khashan and A. Hadi. 2020. Silver nanoparticles and silver ions as potential antibacterial agents. Journal of Inorganic and Organometallic Polymers and Materials, 1-18.

Harrison, R.D. 2005. Figs and the diversity of tropical rainforests. Bioscience, 55(12): 1053-1064.

Hassoun-Kheir, N., Y. Stabholz, J.U. Kreft, R. De La Cruz, J.L. Romalde, J. Nesme, S. J. Sørensen, B.F. Smets, D. Graham and M. Paul. 2020. Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. Science of the Total Environment, 743: 140804.

He, L.X., L.Y. He, F.Z. Gao, D.L. Wu, P. Ye, Y.X. Cheng, Z.Y. Chen, L.X. Hu, Y.S. Liu, J. Chen and G.G. Ying. 2022. Antibiotics, antibiotic resistance genes and microbial community in grouper mariculture. Science of The Total Environment, 808: 152042.

Hepokur, C., İ.A. Kariper, S. Mısır, E. Ay, S. Tunoğlu, M.S. Ersez, Ü. Zeybek, S.E. Kuruca and İ. Yaylım. 2019. Silver nanoparticle/capecitabine for breast cancer cell treatment. Toxicology in Vitro, 61: 104600.

Jadoun, S., R. Arif, N.K. Jangid and R.K. Meena. 2021. Green synthesis of nanoparticles using plant extracts: A review. Environmental Chemistry Letters, 19: 355-374.

Kamath, P and J. Packiyam. 2017. Synthesis of silver nanoparticles from leaf extracts of *Wedelia chinensis* (Osbeck) Merrill and their antimicrobial activity. Journal of Microbiology and Biotechnology Research, 3: 48-53.

Khalil, M.M., E.H. Ismail, K.Z. El-Baghdady and D. Mohamed. 2014. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arabian Journal of Chemistry, 7(6): 1131-1139.

Khan, M.R., M.A. Khan, U. Habib, M. Maqbool, R.M. Rana, S.I. Awan and B.B. Duralija. 2022. Evaluation of the Characteristics of Native Wild Himalayan Fig (*Ficus palmata* Forsk.) from Pakistan as a Potential Species for Sustainable Fruit Production. Sustainability, 14(1): 468.

Kitajima, J., K. Kimizuka and Y. Tanaka. 1999. New dammarane-type acetylated triterpenoids and their related compounds of *Ficus pumila* fruit. Chemical and Pharmaceutical Bulletin, 47(8): 1138-1140.

Kongkham, B., D. Prabakaran and H. Puttaswamy. 2020. Opportunities and challenges in managing antibiotic resistance in bacteria using plant secondary metabolites. Fitoterapia, 147: 104762.

Lalitha, A., R. Subbaiya and P. Ponmurugan. 2013. Green synthesis of silver nanoparticles from leaf extract *Azhadirachta indica* and to study its anti-bacterial and antioxidant property. International Journal of Current Microbiology and Applied Sciences, 2(6): 228-235.

Larsson, D and C.F. Flach. 2021. Antibiotic resistance in the environment. Nature Reviews Microbiology, 1-13.

Le Ouay, B. and F. Stellacci. 2015. Antibacterial activity of silver nanoparticles: a surface science insight. Nano today, 10(3): 339-354.

Awwad, M.A., N.M. Salem and A.O. Abdeen. 2013. Biosynthesis of silver nanoparticles using Loquat leaf extract and its antibacterial activity. Advanced Materials Letters, 4(5): 338-342.

Ma, Y.X., C.Y. Wang, Y.Y. Li, J. Li, Q.Q. Wan, J.H. Chen, F.R. Tay and L.N. Niu. 2020. Eskape Infection Control: Considerations and Caveats in Combating ESKAPE Pathogens against Nosocomial Infections (Adv. Sci. 1/2020). Advanced Science, 7(1): 2070004.

Mahboubi, M. 2021. Iranian medicinal plants as antimicrobial agents. Journal of Microbiology, Biotechnology and Food Sciences, 2388-2405.

Makarov, V.V., A.J. Love, O.V. Sinitsyna, S.S. Makarova, I.V. Yaminsky, M.E. Taliansky and N.O. Kalinina. 2014. "Green" nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae (англоязычная версия), 6(1 (20)): 35-44.

Mishra, V., R. Sharma, N.D. Jasuja and D.K. Gupta. 2014. A review on green synthesis of nanoparticles and evaluation of antimicrobial activity. International Journal of Green and Herbal Chemistry, 3(1): 081-094.

Mulani, M.S., E.E. Kamble, S.N. Kumkar, M.S. Tawre and K. R. Pardesi. 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Frontiers in Microbiology,

10: 539.

Murray, C.J., K.S. Ikuta, F. Sharara, L. Swetschinski, G.R. Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool and S.C. Johnson. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet, 399(10325): 629-655.

Murugan, K., B. Senthilkumar, D. Senbagam and S. Al-Sohaibani. 2014. Biosynthesis of silver nanoparticles using *Acacia leucophloea* extract and their antibacterial activity. International Journal of Nanomedicine, 9: 2431.

Nasser, M., S. Palwe, R.N. Bhargava, M.G. Feuilloley and A.S. Kharat. 2020. Retrospective analysis on antimicrobial resistance trends and prevalence of β-lactamases in *Escherichia coli* and ESKAPE pathogens isolated from Arabian patients during 2000–2020. Microorganisms, 8(10): 1626.

Ogunlaja, O.O., R. Moodley, H. Baijnath and S.B. Jonnalagadda. 2022. Antioxidant activity of the bioactive compounds from the edible fruits and leaves of *Ficus sur* Forssk. (Moraceae). South African Journal of Science, 118(3-4): 1-5.

Osungunna, M.O. 2021. Screening of medicinal plants for antimicrobial activity: Pharmacognosy and microbiological perspectives. Journal of Microbiology, Biotechnology and Food Sciences, 727-735.

Pandit, C., A. Roy, S. Ghotekar, A. Khusro, M.N. Islam, T.B. Emran, S.E. Lam, M.U. Khandaker and D.A. Bradley. 2022. Biological agents for synthesis of nanoparticles and their applications. Journal of King Saud University-Science, 34(3): 101869.

Pendleton, J.N., S.P. Gorman and B.F. Gilmore. 2013. Clinical relevance of the ESKAPE pathogens. Expert Review of Anti-infective Therapy, 11(3): 297-308.

Rajput, N. 2015. Methods of preparation of nanoparticles-a review. International Journal of Advances in Engineering and Technology, 7(6): 1806.

Rauwel, P., S. Küünal, S. Ferdov and E. Rauwel. 2015. A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. Advances in Materials Science and Engineering.

Ravishankar Rai, V. and A. Jamuna Bai. 2011. Nanoparticles and Their Potential Application as Antimicrobials, Science against Microbial Pathogens: Communicating Current Research and Technological Advances. In: Méndez-Vilas, A., Ed., Formatex, Microbiology Series, 3(1): 197-209.

Rout, Y., S. Behera, A.K. Ojha and P.L. Nayak. 2012. Green synthesis of silver nanoparticles using *Ocimum sanctum* (Tulashi) and study of their antibacterial and antifungal activities. The Journal of Microbiology and Antimicrobials, 4(6): 103-109.

Roy, K., C. Sarkar and C. Ghosh. 2015. Plant-mediated synthesis of silver nanoparticles using parsley (*Petroselinum crispum*) leaf extract: spectral analysis of the particles and antibacterial study. Applied Nanoscience, 5(8): 945-951.

Rudramurthy, G.R., M.K. Swamy, U.R. Sinniah and A. Ghasemzadeh. 2016. Nanoparticles: alternatives against drug-resistant pathogenic microbes. Molecules, 21(7): 836.

Sahayaraj, K and S. Rajesh. 2011. Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances, 23: 228-244.

Sarsar, V., K.K. Selwal and M.K. Selwal. 2013. Green synthesis of silver nanoparticles using leaf extract of *Mangifera indica* and evaluation of their antimicrobial activity. Journal of Microbiology and Biotechnology Research, 3(5): 27-32.

Savithramma, N., M.L. Rao, K. Rukmini and P.S. Devi. 2011. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of Chem Tech Research, 3(3): 1394-1402.

Savoia, D. 2012. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiology, 7(8): 979-990.

Sbhatu, D.B., H.T. Tekle and K.H. Tesfamariam. 2020. *Ficus palmata* F orskål (beles adgi) as a source of milk clotting agent: a preliminary research. BMC Research Notes, 13(1): 1-5.

Schmieder, R. and R. Edwards. 2012. Insights into antibiotic resistance through metagenomic approaches. Future microbiology, 7(1): 73-89.

Shumail, H., S. Khalid, I. Ahmad, H. Khan, S. Amin and B. Ullah. 2021. Review on green synthesis of silver nanoparticles through plants. Endocrine,

Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 21(6): 994-1007.

Singh, P., Y.J. Kim, D. Zhang and D.C. Yang. 2016. Biological synthesis of nanoparticles from plants and microorganisms. Trends in Biotechnology, 34(7): 588-599.

Stracy, M., O. Snitser, I. Yelin, Y. Amer, M. Parizade, R. Katz, G. Rimler, T. Wolf, E. Herzel, G. Koren and J. Kuint. 2022. Minimizing treatment-induced emergence of antibiotic resistance in bacterial infections. Science, 375(6583): 889-894.

Tiwari, R., S. Chakraborty, K. Dhama, S. Rajagunalan and S.V. Singh. 2013. Antibiotic resistance–an emerging health problem: causes, worries, challenges and solutions–a review. International Journal of Current Research, 5(7): 1880-1892.

Ugboko, H.U., O.C. Nwinyi, S.U. Oranusi, T.H. Fatoki and C.A. Omonhinmin. 2020. Antimicrobial importance of medicinal plants in Nigeria. The Scientific World Journal.

Zhu, Y., W.E. Huang and Q. Yang. 2022. Clinical perspective of antimicrobial resistance in bacteria. Infection and Drug Resistance, 15: 735.