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Review Article

Green Synthesis of Silver Nanoparticles from Medicinal Plants: A Review of Antimicrobial Potentials Against MRSA

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) represents a major global health challenge due to its resistance to β -lactam antibiotics mediated primarily by the mecA gene encoding penicillin-binding protein 2a (PBP2a). The increasing prevalence of MRSA in hospital, community, and livestock settings necessitates alternative antimicrobial strategies. Silver nanoparticles (AgNPs) have emerged as promising antimicrobial agents owing to their broad-spectrum activity and multi-targeted mechanisms. However, conventional synthesis methods involve toxic chemicals and high energy consumption. Green synthesis using medicinal plant extracts offers an eco-friendly, cost-effective, and biocompatible alternative. This review critically summarizes recent studies on plant-mediated synthesis of AgNPs and evaluates their antimicrobial efficacy against MRSA. Medicinal plants such as Thymus vulgaris, Berberis vulgaris, Foeniculum vulgare, Ananas comosus, Phyllanthus niruri, and Phoenix dactylifera are discussed with respect to nanoparticle characteristics, mechanisms of action, and anti-MRSA potential. Overall, green-synthesized AgNPs demonstrate strong antibacterial and antibiofilm activity with reduced cytotoxicity, highlighting their potential for future clinical and biomedical applications.

INTRODUCTION

MRSA is a virulent form of *S. aureus* that has now become resistant to methicillin and a wide range of other antibiotics. It is now the major cause of various hard-to-treat infections around the world. MRSA came into existence when *S. aureus* acquired a special resistance gene called mecA, encoding an altered protein called PBP2a. This

protein prevents the effective binding of β -lactam antibiotics (which include penicillin, methicillin, and cephalosporins), rendering these drugs all but useless against the bacterium. (1)

Types of MRSA

MRSA Associated with Healthcare (HA-MRSA)

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This super bug, which was first discovered in the 1960s, thrives in hospitals and causes infections there. The ST239 clone, which carries SCCmec III, is the most common multi-drug resistor.

MRSA Associated with the Community (CA-MRSA)

Appeared in the 1990s and affected healthy people who had never been to a hospital. Often carrying the toxic Panton-Valentine leukocidin (PVL) toxin for added virulence, it is associated with strains such as USA400 and USA300 (ST8 with SCCmec IV).

MRSA Associated with Livestock (LA-MRSA)

Emerged in the 2000s, with the ST398 strain predominating in Europe and North America. It's zoonotic, hopping from animals (like pigs) to people, giving the resistance story a farm-to-table dimension. (1)

MRSA can infect skin and soft tissues (boils, abscesses, cellulitis, impetigo) in the community or healthcare settings, and can rapidly escalate to necrotizing fasciitis; bones and joints (osteomyelitis, septic arthritis) either via blood stream or nearby tissues, and can result in reinfection of prosthetics and surgeries; the lungs (pneumonia, usually necrotizing, especially with flu and COPD, mortality is high); bloodstream and heart (septicemia or endocarditis in IV/catheter users and emboli), as well as the urinary tract (UTIs due to catheters and procedures all the way to pyelonephritis or urosepsis), and infrequently, the meninges (inflammation in the brain via the bloodstream). Due to increasing burden of MRSA associated diseases, the need for alternative therapeutic strategies that do not rely solely on conventional drugs.

Because of this necessity, silver has revitalized its position as a strong antimicrobial agent. However, although it was used long before the discovery of modern chemotherapeutic agents, the reemerging relevance of silver is related to the development of AgNPs, which exert increased surface activity, exhibit broad-spectrum antimicrobial activity, and demonstrate strong effectiveness against multidrug-resistant bacteria.

However, traditional methods of chemical and physical nanoparticle synthesis have posed certain concerns regarding toxicity associated with the reagents used, high energy demands, and limited biocompatibility. Therefore, green synthesis using medicinal plants has been given considerable significance owing to their eco-friendliness, safety, and low cost.(2)

Why Green Synthesis of Silver Nanoparticles is Favored :

Green synthesis in nanoparticle production uses plant extracts, microbes, and other natural products rather than toxic chemicals.

Advantages over conventional synthesis:

- Eco-friendly and cost-effective: no toxic reducing or stabilizing agents.
- Biocompatible and safer for medical applications.
- Better stability and controlled particle size owing to naturally occurring capping agents.

Sustainable process using renewable biological materials. Antimicrobial action against MRSA: AgNPs bind to bacterial cell walls, causing membrane disruption, production of ROS, and impairment of proteins and DNA. They can overcome MRSA resistance by bypassing PBP2a and causing multi-targeted damage. (1)



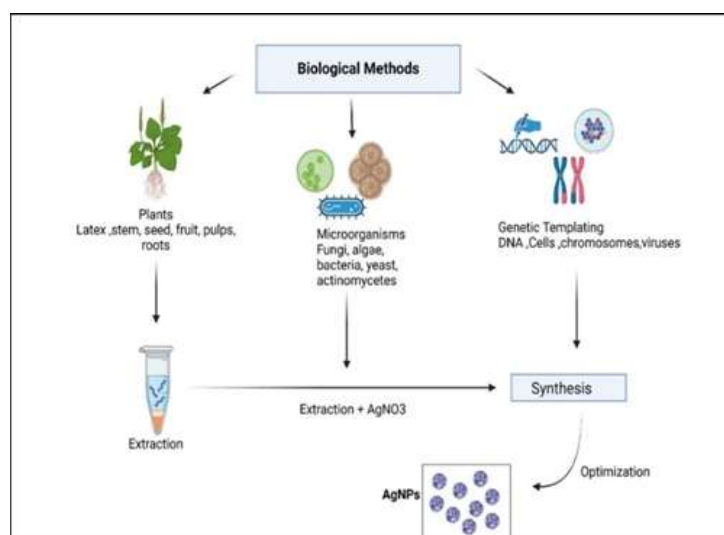


Fig no.1: herbal synthesis of AgNPs

Rationale of the Review :

Despite extensive research on silver nanoparticles, a consolidated review focusing specifically on green-synthesized AgNPs derived from medicinal plants and their efficacy against MRSA is limited. This review addresses this gap by systematically compiling evidence on plant-based AgNPs, their antimicrobial mechanisms, and their advantages over conventional antibiotics.

MATERIALS AND METHODS:

A structured and systematic search approach was conducted for this review. Relevant studies published between 2010 and 2025 were searched through scientific databases, namely PubMed, ScienceDirect, Scopus, Google Scholar, and Web of Science.

Mechanisms of Antibiotic Resistance in MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) exhibits resistance to multiple antibiotics through several well-established mechanisms. The primary determinant is the *mecA* gene, located on the staphylococcal chromosomal cassette *mec* (SCC*mec*), which encodes an altered penicillin-binding protein, PBP2a. This protein has a low

affinity for β -lactam antibiotics, allowing uninterrupted peptidoglycan synthesis even in the presence of these drugs, thereby rendering β -lactams ineffective.. (1)

In addition to *mecA*-mediated resistance, MRSA efficiently forms biofilms, which act as a physical and biochemical barrier against antibiotic penetration and host immune responses. Biofilm-associated cells can tolerate antibiotic concentrations up to 1,000-fold higher than planktonic cells. This process is regulated by genes such as *icaADBC*, *fnbA/B*, *clfA/B*, and global regulators including *agr* and *sarA*. Biofilm formation contributes significantly to chronic, recurrent, and device-associated infections.. (3)

Furthermore, MRSA rapidly acquires resistance determinants via horizontal gene transfer, enhancing its adaptability and pathogenicity. Efflux pumps, enzymatic inactivation, and metabolic dormancy within biofilms further complicate treatment outcomes..(4)

Challenges Posed by MRSA

1) Antibiotic Resistance Profiles

MRSA is resistant to many antibiotics, including the β -lactams (like methicillin) and common antibiotics, such as ciprofloxacin, clindamycin, and doxycycline. (5–7). MRSA can also vary in resistance between hospital-acquired and community-acquired infections, making treatment complicated. For example, HA-MRSA is more resistant to ciprofloxacin than CA-MRSA, while CA-MRSA has greater resistance to clindamycin and chloramphenicol(5)

2) Biofilm formation

MRSA can produce durable biofilms, associated bacterial communities that are encased within extracellular polymeric substances that can protect the bacteria from antibiotics and the immune response of the host 45. Biofilm-associated infections are also known for their persistence and recurrence because biofilm-encased cells can tolerate higher concentrations of antibiotics than planktonic cells.(8,9)

3) Horizontal Gene Transfer and Pathogenicity

Due to horizontal gene transfer, MRSA rapidly acquires and exchanges resistance genes, which allow the development of multidrug-resistant strains. The *mecA* gene located in the staphylococcal chromosomal cassette *SCCmec* is a crucial determinant for methicillin resistance, encoding a penicillin-binding protein, PBP2a, which prevents β -lactam antibiotics from inhibiting cell wall synthesis.

How Green-Synthesized AgNPs overcome these challenges

Green synthesis of AgNPs utilizes environmentally friendly, biologically sourced agents like plant extracts, fungi, or bacteria as the reducing and stabilizing agents. This technique offers certain advantages over the more

conventional chemical or physical synthesis techniques and has solutions to MRSA-associated challenges:

1) Broad-Spectrum Antibacterial Activity

AgNPs synthesized by a green method have shown effective antibacterial activity even against multi-drug-resistant strains such as MRSA. Conjugation of bioactive compounds from herbal sources, including flavonoids, alkaloids, and phenolics, enhances both stability and antimicrobial efficacy.(10,11)

2) Effective against Biofilms

AgNPs can inhibit the formation of biofilms or disrupt preformed biofilms, one of the biggest obstacles in treating MRSA infections. Several studies have proven that green-synthesized AgNPs from different plant or microbial sources have high activity against biofilm layers through compromising their extracellular polymer matrix. (8,9,12)

3) Cytocompatibility and Reduced Toxicity

The bioactive compounds in green-synthesized AgNPs have been reported to lessen cytotoxic effects on mammalian cells. Herbal capping agents, for example, can ensure least damage to the surrounding healthy tissues and thus allow their safe therapeutic applications.(10,13)

4) Multifunctionality

The green-synthesized AgNPs have multifunctional antimicrobial actions that decrease the chances of developing resistance. This multifunctionality is linked to their unique mechanisms of action discussed below.

Mechanisms of Action of General Herbal AgNPs as antimicrobial agents



1) Disruption of Bacterial Cell Membranes

AgNPs disrupt the structural integrity of bacterial cell membranes by interacting directly with the phospholipids and proteins of the membranes. This causes destabilization of membrane integrity, intracellular content leakage, and ultimately, bacterial lysis. In MRSA, such disruption circumvents the resistance provided by the *mecA* gene because the mode of action does not involve cell wall synthesis inhibition.(13,14)

2) Generation of Reactive Oxygen Species (ROS)

AgNPs generate ROS, leading to the induction of oxidative stress. This leads to irreversible damage in bacterial DNA, proteins, and lipids, which prevents bacterial replication and kills both planktonic and biofilm-embedded bacteria.(12,13,15)

3) Interaction with DNA and Proteins

Silver ions released from AgNPs interact with the thiol groups of essential bacterial proteins and

enzymes, thereby interfering with important metabolic pathways. Besides, silver ions can intercalate into bacterial DNA, leading to inhibition of replication and transcription.(8,9)

4) Interference with the Electron Transport Chain

AgNPs disturb the bacterial electron transport chain, which interferes with the production of ATP and causes impairment in energy-dependent bacterial survival mechanisms. This mechanism accounts for broad-spectrum antibacterial action against most Gram-positive and Gram-negative bacteria, including MRSA.(15)

5) Synergistic Effects with Herbal Bioactives

Bioactive molecules like tannins, polyphenols, and flavonoids are incorporated into Herbal AgNPs. These biomolecules act synergistically with nanoparticles to enhance antimicrobial effects. For instance, kaempferol-coated AgNPs have been shown to exert strong antibacterial effects via improved solubility and cellular uptake.)(16)

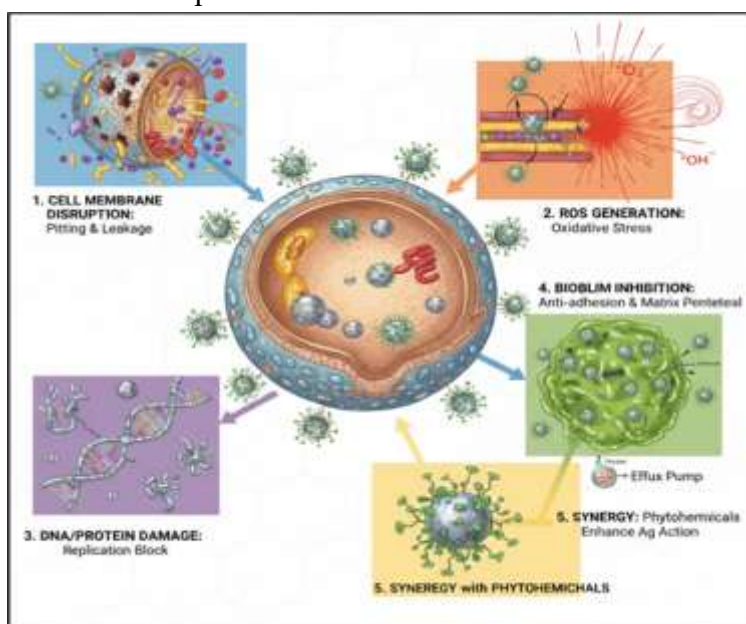


Fig no. 2 : Multi-Target Mechanism of Herbal AgNPs Against MRSA

Antimicrobial potentials of AgNPs synthesized using various herbs against MRSA:

1) Antimicrobial study using AgNPs synthesized using *Berberis vulgaris*

Antimicrobial activity:

Biosynthesized *B. vulgaris* AgNPs exhibit superior activity against Gram-negative and Gram-positive bacteria, such as *Escherichia coli* and *Staphylococcus aureus* respectively, compared to crude extracts or AgNO_3 alone by mechanisms such as thiol-binding to respiratory enzymes, inhibition of phosphate efflux, and membrane disruption, higher efficacy on *E. coli* due to thinner peptidoglycan.

Disk Diffusion: Zones of inhibition increase with concentration, from 1-5 mM AgNPs. 3 mM: ~12-15 mm vs. *S. aureus*, ~15-18 mm vs. *E. coli* (vs. 8-10 mm for extracts; positive controls: gentamicin/streptomycin ~20-25 mm).

MIC: 1 mM fully inhibits *E. coli*, 3 mM for *S. aureus*. Lower than extracts, which require between 5-10 mM for inhibition; no growth in wells at optimal dilution. Overall, AgNPs exhibit a dose-dependent bactericidal action-complete inhibition at 5 mM-facilitated by small size and affinity of sulfur/phosphorus atoms. No side effects noted; alternative to antibiotics in resistant strains.(17)

2) Antimicrobial study using AgNPs synthesized using *Thymus vulgaris*

Antibacterial activity:

The green-synthesized silver nanoparticles (AgNPs) using *Thymus vulgaris* (thyme) leaf extract displayed notable antibacterial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative

(*Escherichia coli* O157:H7, *Pseudomonas aeruginosa*) strains was assayed using disk diffusion and viable cell counts in methylcellulose edible films. Inhibition due to sulfhydryl binding, disruption of DNA, and generation of reactive oxygen species rises accordingly with dose, although Gram-positive bacteria are more sensitive.

Disk diffusion: Zones increase with concentration in disk diffusion with 50, 100, or 150 ppm AgNPs; no inhibition at 50 ppm for *E. coli*, 14 mm at 150 ppm; 10 mm at 100 ppm and 15 mm at 150 ppm for *P. aeruginosa*; 7 mm at 50 ppm, 12 mm at 100 ppm, and 17 mm at 150 ppm for *B. subtilis*; 11 mm at 50 ppm, 15 mm at 100 ppm, and 20 mm at 150 ppm for *S. aureus*, compared to ciprofloxacin zones of 13-22 mm.

Viable Cell Count (Edible Film): Against *E. coli* O157:H7, the edible films at an initial 10^6 - 10^7 CFU/mL showed growth in the control to 11.00 log CFU/mL by 20 hours, while 50 ppm was reduced to 6.40 log by 20 hours, 100 ppm to 5.26 log, and 150 ppm achieved bactericidal effects of 3.25 log after 12 hours incubation at 37°C, with full inhibition at higher concentration by 20 hours, which reveals the potential of food packaging to improve safety and increase shelf life.(18)

3) Antimicrobial study Using AgNPs of Hydroponically Grown *Foeniculum vulgare* (Fennel)

Antibacterial Activity

Hydroponic fennel extract-based AgNPs exhibited significant bactericidal activity in a dose-dependent manner with an MIC/MBC value ≤ 2 against clinical MRSA strains, which is superior compared to the extract and AgNO_3 . Such efficacy was derived from Ag^+ -induced membrane disruption, ROS generation, inhibition of

enzymes, and phytochemical capping that enhanced both its penetration and stability. Various in vitro and BALB/c burn-wound models have established complete MRSA clearance, 100% wound contraction, and full re-epithelialization by day 7. Time-kill assays demonstrated the complete eradication of MRSA in 6 h, which was faster compared to Hydroponic Fennel Extract, ampicillin, fusidic acid, and AgNO₃. These AgNPs-loaded PVP-EC hydrogels, in turn, exhibited 100% wound closure against all tested controls, with undetectable bacterial load, showing great and safe therapeutic potential.

Disc diffusion assays: The zones of inhibition (ZOI, mm) for Hydroponic Fennel Extract AgNPs were 22.2, significantly higher compared to Hydroponic Fennel Extract and AgNO₃, with $p < 0.05$, comparable with the fusidic acid positive control (20-25 mm) and surpassing the ampicillin negative control (0 mm), indicating strong diffusion/penetration.

Minimum inhibitory concentration (MIC): MIC via broth microdilution is 625 µg/mL for Hydroponic Fennel Extract AgNPs, with no visible growth, versus 2000 µg/mL Hydroponic Fennel Extract, 12,500 µg/mL AgNO₃, 6250 µg/mL fusidic acid, and 50,000 µg/mL ampicillin. The MBC was determined as 1250 µg/mL, with no colonies on subcultured agar, versus 4000 µg/mL Hydroponic Fennel Extract, 25,000 µg/mL AgNO₃, 12,500 µg/mL fusidic acid, and 75,000 µg/mL ampicillin, with an MBC/MIC ratio of 2, confirming bactericidal action and low resistance potential.(19)

4) Antimicrobial study using AgNPs synthesized using Ananas comosus:

Antibacterial Activity

The biosynthesized Ananas comosus AgNPs have shown dose- and time-dependent antibacterial activity against one of the major resistant pathogens, MRSA, through mechanisms involving membrane disruption, enzyme inhibition, and ROS generation, enhanced by bromelain and phenolics present in pineapple, proving to be 20% more effective than pure AC extract alone. This positions them as a promising alternative for wound dressings and antimicrobial coatings. Activity assessment was done according to AATCC 100-1999 and Kirby-Bauer standards; serial dilutions were tested qualitatively for inhibition at 25%, 50%, and 100%.

Disk diffusion assays :By using 6 mm discs on Mueller-Hinton agar (MRSA inoculum adjusted to 0.5 McFarland, incubated 24 hours at 37°C), the zones of inhibition average 10.59 mm for AC-AgNPs with peak efficacy at the 3- and 6-hour stirring times, which has the highest diameter observed, outperforming pure AC extract by 2.00 mm while 3.50 mm less than the vancomycin positive control (50 µg/disc; ~14 mm zone); the negative control (5% NaCl or distilled water) did not demonstrate any inhibition. Kruskal-Walli's analysis confirms that significant differences exist ($p < 0.05$) across stirring times, and this shows the optimal duration of synthesis for obtaining potency.

No MIC values are reported; activity is assessed only qualitatively by disk diffusion, and full inhibition at 100% dilution is implied but not quantitated by broth microdilution.(20)

5) Antimicrobial study using AgNPs synthesized using Phyllanthus niruri

Antibacterial study:

Pn-AgNPs demonstrated highly effective, broad-spectrum, dose-dependent bactericidal action,



MBC/MIC ≤ 4 , against a wide set of MDR clinical isolates and ATCC strains, with higher activity compared to the extract and AgNO₃. This was through cell-wall binding, membrane rupture with 4.7-5.1× protein leakage, ROS generation, enzyme/ATP inhibition, DNA/phosphate damage leading to cell lysis. Scanning electron microscopy/TEM analysis confirmed rumpled cells, wall breakage, entry of nanoparticles, and shrinkage of organelles. All the isolates showed multi-drug resistance, hence Pn-AgNPs can be potential candidates against persistent infections.

Well-diffusion test: Pn-AgNPs created clear zones of bacterial inhibition ranging from 10 to 30 mm, showing strong activity against all tested microbes. The controls (AgNO₃, plant extract, and water) showed no activity, confirming that the nanoparticles were responsible for the effect. Most Gram-negative bacteria showed zones of about 10–15 mm, *Enterococcus* and *Enterobacter* around 15–20 mm, *Streptococcus* 20–25 mm, and the most sensitive—MRSA, VRE, and *Burkholderia*—around 25–30 mm.

MIC testing showed that Pn-AgNPs could stop bacterial growth at very low concentrations (10–40 µg/mL), and kill them at 20–80 µg/mL. Sensitive strains like *E. faecalis* and *Enterobacter cloacae* needed only 10 µg/mL, while common pathogens like *E. coli* and *P. aeruginosa* required 20 µg/mL. More resistant bacteria—*S. aureus*, *Streptococcus*, *Salmonella typhi*, MRSA, VRE, and CRE—needed 40 µg/mL. Overall, these MIC values were 2–4 times lower than the plant extract alone, showing that the plant metabolites on the nanoparticle surface helped them enter the cells

better and generate stronger ROS-based killing.(21)

6) Antimicrobial study using AgNPs synthesized using Phoenix dactylifera (date palm) seed extract.

Antibacterial study:

The study provides assessment of bactericidal potential against MRSA ATCC 43300 using AgNPs synthesized using Phoenix dactylifera (date palm) seed extract. AgNPs exhibited dose-dependent antibacterial activity through mechanisms involving disruption of the bacterial cell membrane, leakage of intracellular components, damage to cell walls, and eventual lysis, as verified by SEM and HR-TEM analyses.

Key Metrics: Minimal Inhibitory Concentration (MIC): 10.67 ± 0.94 µg/ml. Lowest concentration that inhibits 99% visible bacterial growth.

Minimal Bactericidal Concentration (MBC): 17.33 ± 1.89 µg/ml (Lowest concentration that kills 100% of the original bacterial population.)

Well-diffusion tests : Zone of inhibition is from 11 mm at 7.8 µg/ml, it enlarged progressively to about 13 mm at 15.6 µg/ml, 15 mm at 31.25 µg/ml, 17 mm at 62.5 µg/ml, 19 mm at 125 µg/ml, 22 mm at 250 µg/ml, reaching a maximum of 24 mm at 500 µg/ml, confirming robust inhibition and membrane disruption as visualized by SEM and HR-TEM, where treated cells showed wrinkled, damaged walls, cytoplasmic leakage eventual lysis. Overall, these findings position the AgNPs as a promising, eco-friendly option for combating MRSA-associated infections in medical devices.(10)

Comparative study of antimicrobial potentials

Medicinal plant	Extract used	Size of AgNPs (nm)	MIC/MBC Against MRSA	Zone of inhibition	Key Antibacterial mechanism
Berberis vulgaris	Leaf / root aqueous extract	~30 (5–50)	MIC: 3 mM (<i>S. aureus</i>)	12–15	Thiol-binding to respiratory enzymes, inhibition of phosphate efflux, membrane disruption; dose-dependent bactericidal action (16)
Thymus vulgaris	Leaf extract	~44 (average)	-	11–20 (50–150 ppm)	Sulphydryl binding, ROS generation, DNA disruption, reduction in viable cell counts (17,21)
<i>Foeniculum vulgare</i> (hydroponic)	Whole plant extract	~35	MIC: 625 µg/mL; MBC: 1250 µg/mL	22.2	Ag ⁺ -induced membrane disruption, ROS production, enzyme inhibition; complete MRSA eradication and wound healing (18)
Ananas comosus	Peel extract	~12	-	10.59	Membrane disruption, enzyme inhibition, ROS generation; enhanced activity due to bromelain and phenolics (19)
Phyllanthus niruri	Leaf extract	~20	MIC: 10–40 µg/mL; MBC: 20–80 µg/mL	25–30	Cell-wall binding, membrane rupture, ROS generation, ATP/DNA/protein damage; strong antibiofilm action(20)
Phoenix dactylifera	Seed extract	14–30	MIC: 10.67 ± 0.94 µg/mL; MBC: 17.33 ± 1.89 µg/mL	11–24	Cell membrane disruption, intracellular leakage, cell wall damage and lysis(9)

Limitations of Green-Synthesized AgNPs

Although green synthesis offers significant advantages, several limitations remain. These include batch-to-batch variability due to differences in phytochemical composition, difficulty in large-scale production (22), lack of standardized synthesis protocols, limited long-term toxicity data, and the absence of extensive clinical trials. Addressing these challenges is essential for successful clinical translation.(23)

FUTURE PERSPECTIVES AND CLINICAL TRANSLATION

Future research should focus on standardizing green synthesis protocols, comprehensive toxicological evaluation, and formulation of AgNPs into clinically applicable delivery systems

such as wound dressings, coatings, and hydrogels.(24) Combination therapies involving AgNPs and conventional antibiotics may further reduce resistance development. Regulatory approval and well-designed clinical trials will be critical for translating green-synthesized AgNPs into effective anti-MRSA therapeutics.(25)

DISCUSSION AND CONCLUSION:

The review demonstrates that green synthesis of silver nanoparticles from medicinal plants is a potent alternative strategy to combat MRSA, which exerts resistance to antibiotics via modification in PBP2a, production of β -lactamase enzymes, efflux pumps, and biofilm formation. Plant extracts rich in phytochemicals, acting as natural reducing and stabilizing agents, give AgNPs enhanced antimicrobial activity and better



biocompatibility compared to AgNPs synthesized chemically.

AgNPs derived from *Berberis vulgaris*, *Thymus vulgaris*, *Foeniculum vulgare*, *Ananas comosus*, and *Phyllanthus niruri*, *Phoenix dactylifera* showed high anti-MRSA effects by membrane disruption, ROS generation, inhibition of DNA/protein synthesis, and suppression of biofilm. Smaller and stable nanoparticles showed higher activity.

Although promising, there are variations in synthesis methodologies and limited in-vivo data; more standardized protocols and toxicity assessments will be required. Overall, green-synthesized AgNPs represent a promisingly sustainable approach with strong potency and multi-mechanisms against MRSA for future applications.

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