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*Review Article*

# Elucidating the Role of Plant Extracts Mediated Gold Nanoparticles as Smart Antimicrobials: Two-Way Attack

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Pathogenic bacteria remain the primary health concern even after developing a broad spectrum of antibiotics. The emergence of drug-resistant strains increased mortality, perhaps resulting in a never-ending future pandemic. The researchers are currently concentrating their efforts on nanotechnology-based therapies to counteract resistance. The present review focuses on the antimicrobial characteristics of plant-mediated gold nanoparticles (Au NPs). First, the methodology and importance of green synthesis are highlighted. Variability in NPs characterization methods was identified, with dynamic light scattering, zeta potential, and thermogravimetric analysis limited to a few investigations. Second, the Au NPs synthesized using different plant extracts were found to be broad-spectrum antimicrobial agents against *Pseudomonas aeruginosa*, *Escherichia coli*, *Cryptococcus neoformans*, *Candida glabrata*, *Aspergillus niger*, etc. The lowest minimum inhibitory concentrations range (1.95–15.62 $\mu$g/mL) was observed with Au NPs synthesized using *Thymus vulgaris* extract against *Staphylococcus aureus*, *P. aeruginosa*, *Bacillus subtilis*, and *E. coli*. The effect was more pronounced with smaller NPs (<10 nm). The activity of Au NPs might be mediated through a two-way attack which includes nanoparticles' diverse mechanisms like reactive oxygen species generation, etc., and surface-attached phytocompounds such as flavonoids, alkaloids, phenolics, terpenoids, tannins, etc. The futuristic role of nanotechnology-based interventions in managing microbial infections is imperative. Synergistic interactions of Au NPs with antibiotics, toxicity profiling, stability, and bioavailability could be major areas of NPs research.

## 1. Introduction

Bacterial infections continue to be a leading cause of morbidity and mortality. The emergence of multidrug-resistant bacterial strains and biofilm-associated illnesses necessitates the development of new antimicrobials [1, 2]. Every year, more than 2 million antibiotic-resistant illnesses occur in the United States, resulting in 23,000 fatalities, according to the US Centers for Disease Control and Prevention [3]. Antibiotic-resistant pathogens are responsible for 25,000 deaths yearly in the European Union [4]. Antibiotic misuse and abuse greatly favor resistance among bacterial pathogens which is a prime cause of the lack of efficacy of existing antimicrobials. Unfortunately, more than 70% of all harmful microorganisms resist at least one standard antibiotic [5]. In addition, biofilm formation also makes it more challenging to treat various infections [6]. The development of new antimicrobials is the need of the hour; one of the most promising candidates in this setting could be the nanoparticles (NPs). Due to their high surface area-to-volume ratio and unique

features, nanoscale materials have emerged as potential antimicrobial agents [7, 8]. The antimicrobial activity of NPs such as Ag, Au, Pt, MgO, CuO, Al, CdS, $TiO_2$, $Cr_2O_3$ NPs, and others has been reported against several drug-resistant bacterial and fungal species [9–17]. In continuation, NPs can replace antibiotics for treating bacterial infections caused by *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* [18].

Toxic substances used in physical and chemical procedures may reside in the generated NPs, creating a concern for the environment and limiting their application in medicine [19]. So, now researchers are shifting toward synthesizing NPs using a green approach. Plant extracts, microorganisms, and other natural products are commonly used in the green synthesis of NPs [20–22]. Most of the NPs have been synthesized using plant materials, including leaves, flowers, bark, etc. Instead of using chemical reagents, all of these plant parts include polyphenols and proteins that can reduce metal ions into their lower valence states [23, 24]. Subsequently, bacteria, algae, and fungi are also employed because they have high concentrations of the reductase enzymes that transform the ionic form into its nano form [25]. Furthermore, the ease of growing and controlling the shape and size of synthesized NPs can drastically lower the cost of producing enormous amounts [26].

The processes of NPs synthesis from various sections of plants are cost-effective, environmentally benign, and simple [27, 28]. In addition, plant extracts also offer the benefit of reducing metal ions in less time [28]. Several nanoparticles, such as $TiO_2$ using *Azadirachta indica* [29], CuO using *Ocimum tenuiflorum* [30], and ZnO using *Aloe vera* [31] synthesized using the green approach were found to be effective antimicrobials. Among nanoparticles, metal and metal oxide NPs had remarkable biomedical activity [27]. Metallic NPs have also been shown to be efficient against diverse pathogens and their selectivity for bacterial strains [32]. Further, metallic NPs have been used in conjunction with antibiotics to overcome antibiotic resistance and improve the latter's efficacy [33, 34]. Different NPs use diverse mechanisms to inhibit pathogens, and metallic NPs, in particular, have been found to penetrate the bacterial cell wall and create pores on the surface of the membrane, causing free radical production that damages the cell membrane [35]. Subsequently, NPs' ions can inhibit enzyme production and produce reactive oxygen species (ROS). In addition, it has been shown that the transcription of DNA is affected [36].

Au NPs have many benefits over other NPs, and some of the advantages include easy synthesis using several methods [37]; exclusive chemical, physical, and optical properties because of size and shape [38]; a high surface area that provides compact drug loading and easily can reach to the target site through blood flow [39], and importantly, noncytotoxic to the normal cells [40]. Suchak et al. [41] reported that Au NPs were active at higher concentrations (0.005 M) against Gram-positive and negative bacterial strains. Likewise, the broad-spectrum antibacterial potential of Au NPs is also documented by other researchers [42]. Keeping in view the importance of Au NPs, the antimicrobial potential of plant-mediated Au NPs has been reviewed. The synthesis of Au NPs utilizing a plant-mediated green technique is thoroughly covered in this article, along with their broad-spectrum antibacterial activity. The significance of particle size, plant selection, and several other aspects have all been described in detail. Early reviews focused only on synthesis techniques or antibacterial potential; however, more thorough investigations highlighting the different research gaps are still necessary. The antibacterial mechanism of Au NPs is outlined in light of existing literature, and the mode of action of phytoconstituents against microbial pathogens is also illustrated.

## 2. Green Synthesis of NPs: Factors Affecting the Formation of NPs

Metallic NPs applications in healthcare have drawn a lot of attention. Scientists for synthesizing NPs have devised several methods, but their use is constrained by harmful chemicals, high-energy demands, and the release of toxic byproducts [43, 44]. For overcoming the drawbacks of physicochemically produced NPs, biological or green synthesis methods have proven to be effective solutions. Compared to other methods, NPs synthesized by biological means have enhanced characteristics for use in biomedical applications [45]. Green synthesis is an economical, easy, eco-friendly, and efficient approach to forming NPs through microorganisms and plants [46–48]. Microorganism-derived NPs are overwhelmed by plant-derived NPs owing to their single-step, nonhazardous process. Another downside of the microbial synthesis approach is time-consuming and expensive downstream processing [45, 49]. The biological synthesis precursors, the process, and the factors affecting NPs synthesis and characterization detail are depicted in Figure 1.

During the NPs synthesis process, it is crucial to consider the selection of a solvent, a safe reducing agent, and a nontoxic component for NPs stabilization [51]. Additionally, several parameters, including pH, salt content, incubation time, temperature, centrifugation, and reaction time, must be optimized to increase the effectiveness, size, and morphology of NPs generated using a green approach [50, 52]. To prevent the development of large aggregated NPs, the concentration of the reducing agent must be optimized. Thermal heating needs to be regulated simultaneously during synthesis; it can denature the bioactive molecules associated with the capping and stabilization of NPs. Altering the concentration of bioactive chemicals can improve the reaction rate. Subsequently, there are several drawbacks, such as the complexity of identifying the plant bioactive components responsible for the synthesis of NPs that influence their therapeutic effects [52, 53].

Additionally, temperature is one of the most significant factors for synthesizing NPs [53]. At higher temperatures (60°C), Mountrichas et al. [54] showed a faster and more uniform synthesis of NPs. The influence of pH on the synthesis of Ag NPs was studied by Gontijo et al. [55], who observed different-sized NPs (5–249 nm) at pH values between 2 and 9. The authors also observed fewer aggregated NPs at a pH <3 and >7. On the other hand, Traiwatcharanon

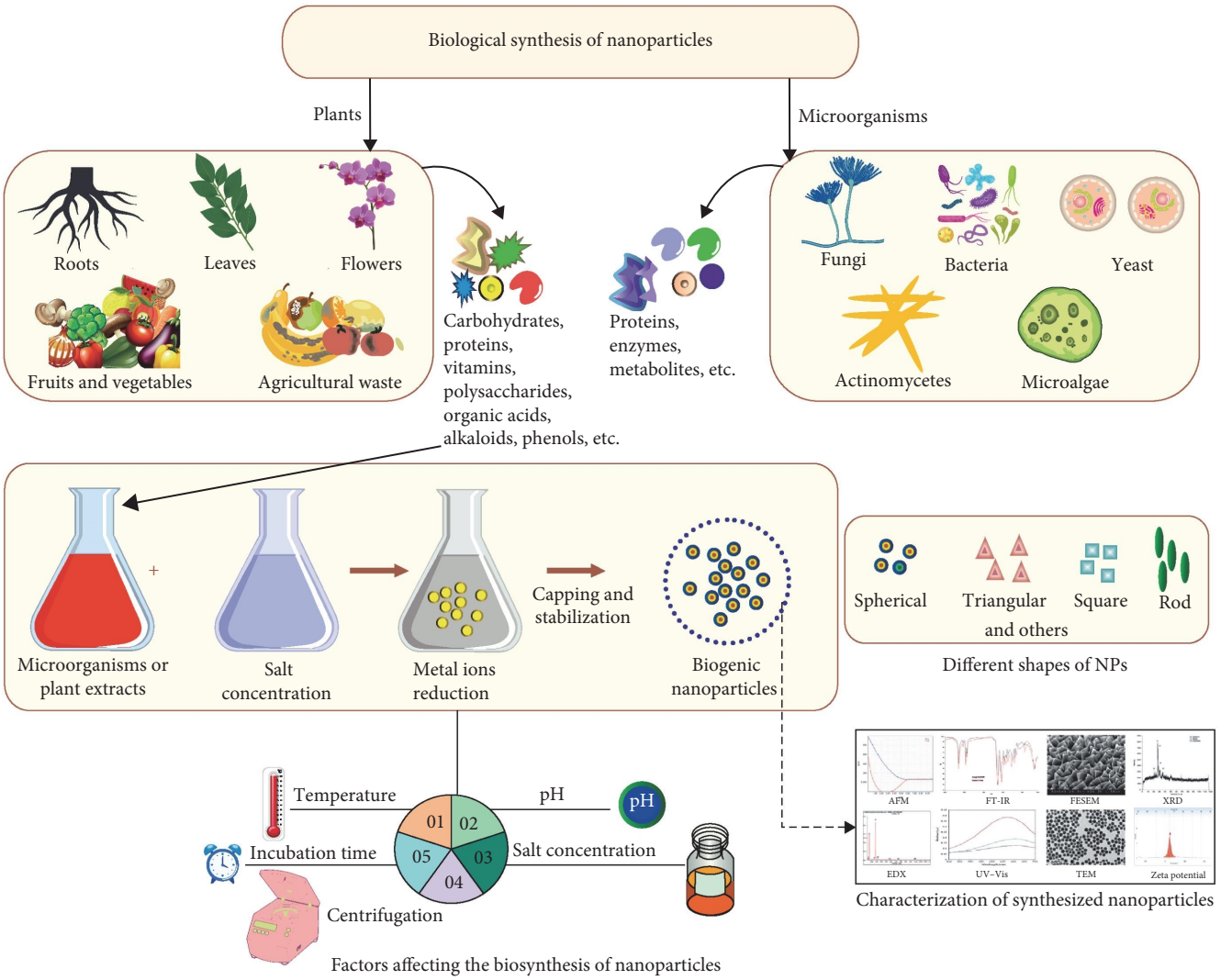


FIGURE 1: Green synthesis process, factors affecting NPs formation, and characterization of metallic nanoparticles. Reproduced from Ali et al. [50] with modifications under Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

et al. [56] used UV–Vis spectroscopy and transmission electron microscopy to study the impact of pH on the nature of Ag NPs. The authors noticed that when Ag NPs were synthesized in an acidic medium as opposed to a basic one, smaller size particles were produced. Optimizing the reducing agent's concentration will help prevent the formation of large aggregated NPs. Ahmad et al. [57] used different concentrations of Au(III) (0.51–4.055 mM) for the synthesis of Au NPs using aqueous *Elaeis guineensis* (palm oil), where Au NPs were not formed at a concentration <1.53 mM. According to the authors, the reduction reaction is too weak because of the extremely low Au(III) concentration, which needs validation. The effects of pH, reaction medium, and reaction time on the synthesis of Ag NPs from mangosteen (*Garcinia mangostana*) leaf extract were investigated by Veerasamy et al. [58]. According to the authors, primary conditions promote the formation of Ag NPs, while acidic solutions hinder this process. Similarly, the author reported more NPs production at longer reaction times. At pH 4, the authors reported large-sized NPs, but at pH 8, they reported small-sized, extensively dispersed NPs. Different parameters need to be optimized for the synthesis of NPs as they affect reaction rate, size, shape, and biological potential.

## 3. Green Synthesis of Au NPs

Au NPs can be prepared by several physical and chemical approaches [43, 44]. However, their usage in biomedical applications is confined due to high energy consumption and the formation of hazardous byproducts [48]. Green synthesis, which includes plants, fungi, bacteria, actinomycetes algae, etc., could be utilized as a cost-effective, biocompatible, and eco-friendly approach [27, 59–62]. Diverse phytocompounds, like alkaloids, terpenoids, phenolics, etc., assist in metal ion reduction and stabilization during green synthesis [63]. The liquefied Au ions get condensed into Au NPs with the help of biological agents in the green approach. This approach led to various unique chemical and physical properties of Au NPs, such as a large surface-to-volume compared to bulk material with the same composition, with

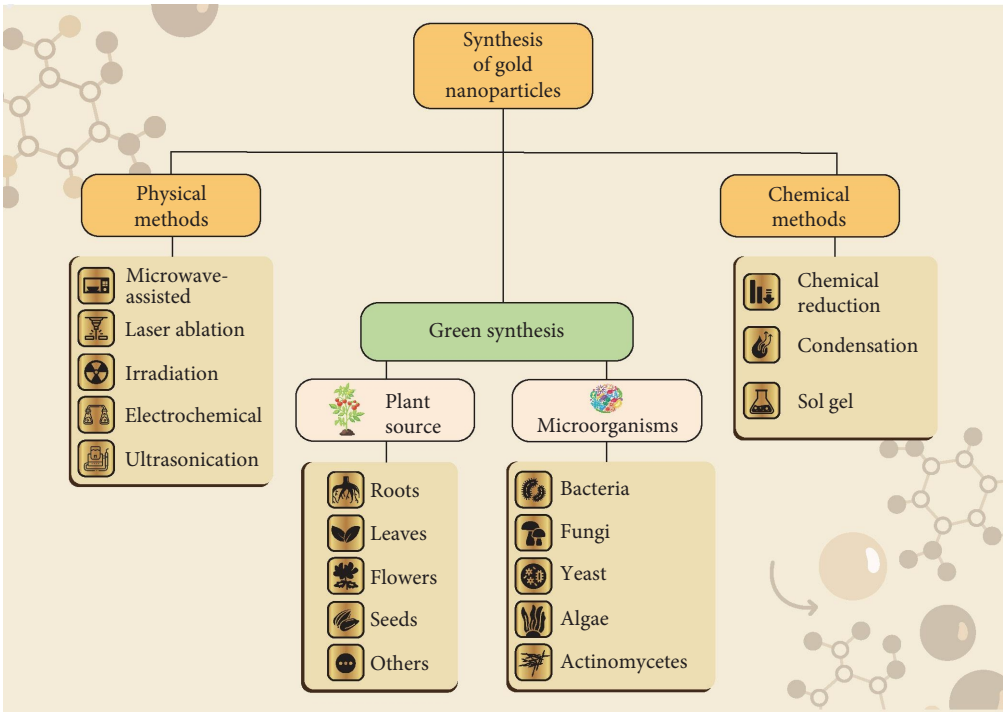


Figure 2: A brief outline of various methods used to synthesize Au NPs.

diverse applications like catalytic, drug delivery, anticancer, antibacterial, etc. [62]. The green approach can control the morphology (shape and size) of Au NPs that play a substantial role in diverse applications [64, 65].

To synthesize Au NPs, Au metal precursor solution is mixed with plant extract, a reducing and stabilizing agent [66]. Au NPs' synthesis process consists of a three-phases, reduction, nucleation, and crystal nuclei growth [67, 68]. In the first phase (reduction), the precursor gets reduced while Au atoms continuously increase [69]. In the second phase (nucleation), the Au atoms form crystal nuclei when they reach the critical super-saturation, and the concentration of Au atoms starts to decrease [70, 71]. Crystal nuclei stop growing when the concentration falls below saturation, resulting in the formulation of pure Au NPs [72, 73]. An overview of various synthesis methods and the plant-mediated synthesis process is highlighted in Figures 2 and 3, respectively.

Plant extracts were used in several investigations to generate Au NPs, although the precise reaction mechanism is still unknown [74, 75]. Using a putative mechanism, Ahmad et al. [76] described how an extract from the leaves of *E. guineensis* might reduce $Au^{3+}$ and stabilize Au NPs. The authors claim that most phenolic compounds, such as caffeic, ferulic, protocatechuic, and gallic acids, contain hydroxyl and carboxylic groups in their structure, which trigger the metallic ions to transform into metal particles [77]. Due to their exceptional binding potential, phenolic compounds (gallic acid) convert $Au^{3+}$ into gold atoms by releasing electrons. Quinones and Au NPs are formed during the oxidation of gold intermediate complexes generated during the reduction reaction. Quinones create repulsive forces that inhibit aggregation and improve colloidal stability as they bind to the surfaces of Au NPs through carbonyl groups. In the literature, it has also been reported that quinones, which bind to Au NPs via negatively charged carbonyl groups, can serve as bio-capping agents similar to gallic acid. Catechin oxidation reduces Au ions, resulting in semiquinone, which is further oxidized to create stable quinones. Due to hydroxyl oxidation, stable quinones produce negatively charged carbonyl groups that cover reduced gold atoms [78]. During the conversion of enol to quinonoid, the generated electrons reduce $Au^{3+}$ ions to gold atoms. The mechanistic component of creating NPs via environmentally friendly techniques requires more study [46].

*3.1. Pros and Cons of Various Synthesis Methods.* Several chemical, physical, and biological techniques have been used to create Au NPs. Some chemical processes utilized to create Au NPs include Turkevich, Brust, seed-mediated development, and digestive ripening [79]. The Turkevich method is quite simple and reproducible for producing spherical particles between 10 and 30 nm, but as the size increases, they become less spherical [80]. The burst approach produced heat and air-stable NPs and used organic solvents that were not water-miscible, limiting these materials' applicability in biological systems [81]. Chemical processes have several benefits, including low cost, high yield, size controllability, and heat stability. These techniques have some drawbacks, including low purity, the usage of hazardous compounds, and organic solvents. Chemical techniques also have inherent limits, such as environmental issues and biocompatibility. Therefore, physical methods have numerous benefits, including rapid speed, no use of hazardous chemicals, and uniform size and shape. Limitations of this technique include high costs,

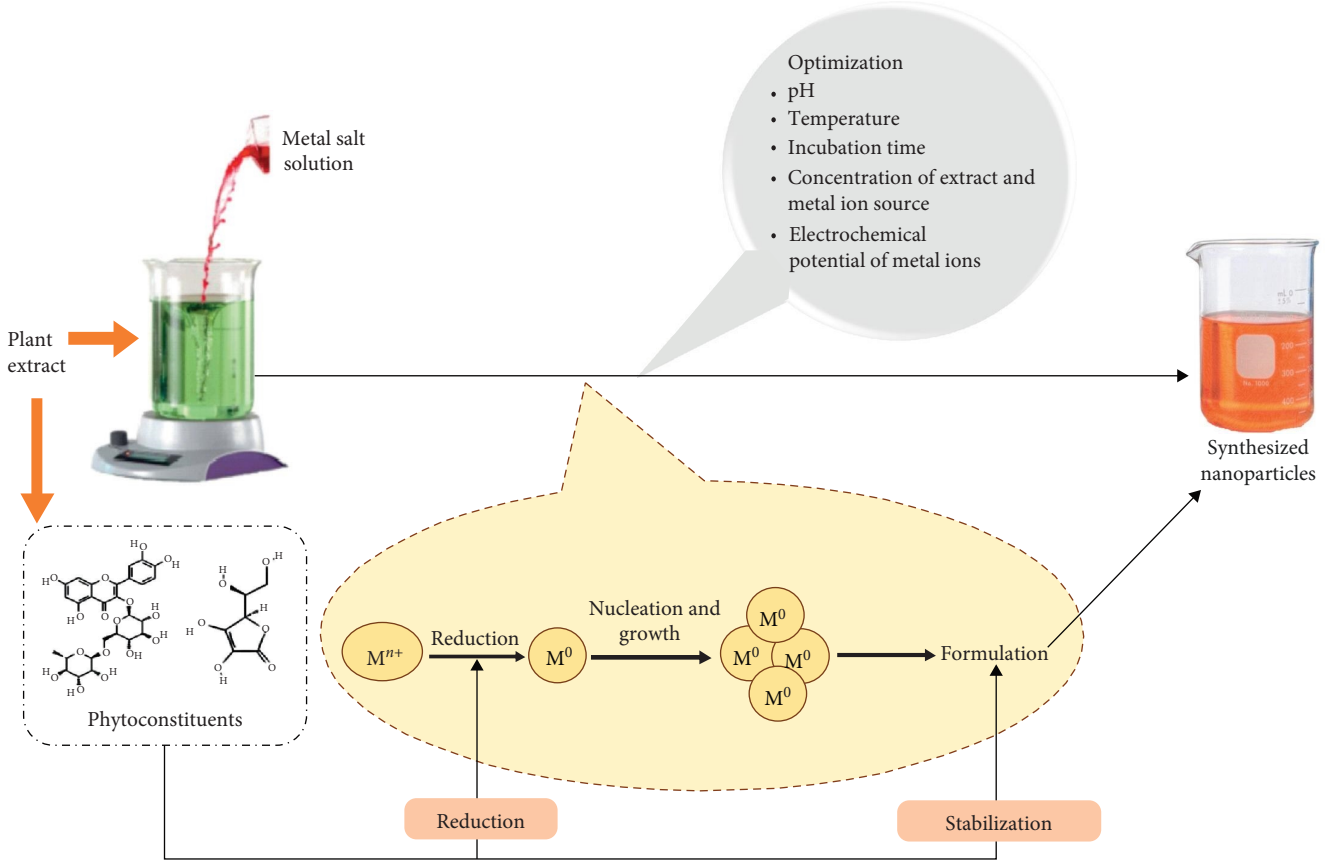


FIGURE 3: An overview of the green synthesis of metal NPs using plant extracts. Reproduced from Balkrishna et al. [48] under Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

radiation exposure, high energy requirements, temperature, and pressure requirements, decreased stability, changing surface chemistry, and altered physicochemical features of NPs [82].

Au NPs can now be produced biologically in a way that is easy, safe, environmentally friendly, nontoxic, and biocompatible [79]. There have been reports of employing fungi, plants, algae, and bacteria to synthesize Au NPs. Microbial methods necessitate time-consuming and expensive downstream processing. Additionally, because removing mycelia from culture filtrates is challenging, so fungus biomass preparation for the synthesis process necessitates cautious measures. Also, some species of microorganisms are pathogenic [83–85]. Algae require a long time to grow, making the process tedious and time-consuming. However, synthesizing Au NPs from algal biomass is very simple and easy. Plant-mediated synthesis is an eco-friendly approach and has several advantages over other methods. The drawback of employing plants for Au NPs formation is that it is challenging to identify the reactive components because plant biomass includes diverse components [86–88].

## 4. Antimicrobial Profile of Au NPs

Since the advent of nanotechnology, many Au NPs have been deployed in various fields because of their exceptional value. Because of its inertness and nontoxicity, gold can conjugate with multiple metals to generate highly stable alloys [89]. Due to their excellent therapeutic effectiveness and negligible side effects, Au NPs have recently piqued the interest of researchers intensely in treating clinically important bacteria, fungi, and parasites. Lately, the foremost concerns that imperil society's health are emerging diseases and the surge in antibiotic resistance among microbes via different unknown pathways [90]. A range of studies has indicated that various green synthesized plant-based Au NPs played an essential role in protecting against various microbes.

In this context, *Actinidia deliciosa* fruit synthesized Au NPs damaged the cell membrane of *P. aeruginosa*, as evidenced by transmission electron microscopy [91]. Emmanuel et al. [92] revealed the remarkable antimicrobial effect of *Justicia glauca* leaves-mediated Au NPs (0.78–50 $\mu$g/mL) against *Escherichia coli*, *Micrococcus luteus*, *S. aureus*, *Streptococcus mutans*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *P. aeruginosa*, and *Candida albicans* with minimum inhibitory concentrations (MIC) ranged between 6.2 and 25 $\mu$g/mL, where the lowest MIC value was observed against *P. aeruginosa*. However, the *Rhazya stricta*-mediated Au NPs displayed moderate activity against *B. subtilis* and *E. coli* with a MIC value of 50 $\mu$g/mL [93]. Additionally, Au NPs synthesized using the seeds of *Embelia ribes* exhibited strong activity against *E. coli* and *S. aureus* with a zone of inhibition range between 22 and 34 mm,

where tetracycline (15 mg/mL) was used as a reference (17 and 11 mm against *S. aureus* and *E. coli*, respectively) [94]. In addition, *Indigofera tinctoria* leaf-mediated Au NPs, when investigated against *Bacillus pumilus*, *Pseudomonas* sp., *E. coli*, *S. aureus*, *Aspergillus fumigatus*, and *Aspergillus niger* exhibited inhibition of 10–30 mm. This efficacy might be due to the surface-functionalized biologically active compounds of *I. tinctoria*, such as alkaloids, flavonoids, and saponins [95]. Likewise, *Stereospermum suaveolens* (root bark) capped Au NPs at 50 $\mu$L showed inhibition (10–25 mm) against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Aspergillus flavus*, and *Aspergillus nidulans* [96].

In another study, *Origanum vulgare* aerial parts mediated Au NPs (1 mL) were effective against *Salmonella enteritidis*, *Listeria monocytogenes*, *E. coli*, *S. aureus*, and *C. albicans* by using disc diffusion assay with inhibition zone diameter ranging between 8 and 28 mm [97]. Moreover, Hamelian et al. [98] found that *Thymus vulgaris* (Thyme) Au NPs inhibited *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus* with MIC and MBC ranged between 3.9–15.62 and 15.62–62.5 $\mu$g/mL, respectively. The study also unveiled that the polyphenolic compounds of Thyme leaves could be responsible for inhibiting microorganisms. Subsequently, Au NPs synthesized using *Alpinia nigra* leaves (400 $\mu$g/mL) also inhibited both bacterial (*B. subtilis*, *E. coli*) and fungal pathogens (*C. albicans*) with IZD (inhibitory zone diameter) 15–18 mm when compared with standard antibacterial chloramphenicol (IZD 28–30 mm at 30 $\mu$g) and nystatin (50 $\mu$g), a standard antifungal drug with IZD 30 mm [99]. Also, the antibacterial activity of biosynthesized (using *Nigella arvensis* leaves) Au NPs was investigated against *B. subtilis*, *Staphylococcus epidermidis*, *P. aeruginosa*, *E. coli*, *S. aureus*, and *Serratia marcescens*. *N. arvensis* Au NPs displayed mild activity with inhibition zone diameters of 11, 10, and 11 mm, respectively, against *E. coli*, *S. aureus*, and *B. subtilis*, while showed complete inhibition against *S. epidermidis*. However, it showed an IZD of 6 mm against both *S. marcescens* and *P. aeruginosa*. Additionally, the MIC of Au NPs against all tested strains ranged between 62.5 and 250 $\mu$g/mL. It was hypothesized that metal NPs and secondary metabolites found in plant extract worked together to damage bacterial cell walls, causing cell respiration to be disrupted [100].

Subsequently, Dhayalan et al. [101] elucidated the antimicrobial status of Au NPs prepared using *Coleus forskohlii* roots (RECo-GNPs) against *M. luteus* and *Proteus vulgaris* by well diffusion method. The RECo-GNPs (250–1,000 $\mu$g/mL) showed mild antibacterial action with a 9–15 mm zone of inhibition and 1–11 mm against *P. vulgaris* and *M. luteus*, respectively. In contrast, positive control, tetracycline (30 $\mu$g/mL) showed IZD 19 and 15 mm, respectively. In another study, *Amomum villosum* fruits mediated Au NPs (45 $\mu$g/disc) demonstrated antibacterial activity with IZD of 17.66 and 18 mm against *S. aureus* and *E. coli*, respectively, where neomycin (30 $\mu$g/disc) was used as a positive control (IZD 15.33 and 15 mm, respectively) [102]. Concurrently, *Bauhinia purpurea* leaves mediated Au NPs (50 $\mu$L) were also ound to be effective antimicrobials against *B. subtilis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *A. flavus*, and *A. nidulans* when evaluated using the agar well diffusion method with IZD ranging between 8 and 12 mm [103]. *G. mangostana* fruit rind-mediated Au NPs exhibited activity only against *Pseudomonas* sp. with 11 mm IZD in the disc diffusion method. However, they were inactive against *Staphylococcus* sp., *Bacillus* sp., and *Klebsiella* sp. [104].

Besides this, Au NPs synthesized using *Peganum harmala* (leaves and seeds) were investigated for antibacterial potential in agar and liquid media against *E. coli* and *S. aureus*. The seed extract-mediated NPs were found inactive in agar media up to 200 $\mu$g/mL while, in liquid media, they showed 15.5% and 10% inhibition against *S. aureus*, and *E. coli*, respectively. On the other hand, in agar media, leaves mediated Au NPs showed IZD 25 and 30 mm against *E. coli* and *S. aureus* at 200 $\mu$g/mL, while in liquid media, 20% and 91.8% inhibition was observed against *E. coli* and *S. aureus*, respectively [105]. Also, the Au NPs (90 $\mu$g/mL) prepared using *Chenopodium formosanum* shell and the positive control, streptomycin (40 $\mu$g/mL), displayed 84.44% and 98.45% inhibition, respectively, against *E. coli*, whereas 59.23% and 99.83% inhibition were observed against *S. aureus* [106]. In another study, *Dillenia indica* leaf synthesized Au NPs (1.5 mg/mL) exhibited IZD 25.5 and 28 mm against *S. aureus* and *E. coli*. However, the standard ciprofloxacin showed IZD 20 and 23 mm against *S. aureus* and *E. coli* [107]. Au NPs prepared using *Musa acuminata* flowers, when evaluated against *P. aeruginosa*, *S. aureus*, *Proteus mirabilis*, *E. coli*, *Salmonella typhi*, *Enterococcus faecalis*, and *K. pneumoniae*, were found active with IZD ranging between 7 and 11 mm [108]. Simultaneously, *Coleus aromatics* leaves mediated Au NPs coated cotton fabric, when investigated for antibacterial efficacy against *S. epidermidis* (Gram-positive) and *E. coli* (Gram-negative) strains, exerted remarkable sensitivity with IZD 22 and 27 mm, respectively [109].

Green synthesized Au NPs prepared using *Clerodendrum inerme* leaves were evaluated against various pathogens (*E. coli*, *B. subtilis*, *Klebsiella* sp., *S. aureus*, *A. flavus*, *A. niger*, and *Trichoderma harzianum*) and also for antibiofilm activity against *S. aureus*, *B. subtilis*, *E. coli*, and *Klebsiella* sp. in comparison to the standard antibacterial (Cephradine) and antifungal (Terbinafine hydrochloride) drugs. The resultant Au NPs (at 125–1,000 $\mu$g/mL) showed significant ($p < 0.001$) activity against all the tested strains concerning their respective standards. Moreover, the nanoparticles revealed substantial ($p < 0.001$) anti-biofilm activity. Thus, it can be said that *C. inerme* leaves biosynthesized Au NPs demonstrated microbicidal activity attributed to surface-functionalized alkaloids, flavonoids, saponins, phenolics, anthraquinones, terpenoids, and tannins [110].

The moderate antimicrobial effect of *Mangifera indica* seeds mediated Au NPs (10–50 mg/mL) was observed against fourteen microbial strains, *Bacillus cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *Corynebacterium rubrum*, *P. aeruginosa*, *Salmonella typhimurium*, *K. pneumoniae*, *Cryptococcus neoformans*, *Candida albicans*, and *C. glabrata* as well as fungal clinical isolates (No.12, 15, 22) with a zone of inhibition ranging from 5 to 10 mm [111]. Also, a study showed that Au NPs synthesized using *Solanum nigrum* leaves (1.6–100 $\mu$g/mL) exerted antimicrobial efficacy against *E. coli* with IZD of 19.2–20 mm [112].

Boomi et al. [113] showed that the *Acalypha indica* leaves mediated Au NPs (10 $\mu$g/mL) strongly inhibited *S. epidermidis* (IZD 31 mm) as compared to *E. coli* (26 mm). A brief overview of the antimicrobial potential of Au NPs synthesized using various plant extracts is high. The *Cynodon dactylon* (plant) biosynthesized Au NPs exhibited antimicrobial efficacy against *Enterobacter cloacae*, *Staphylococcus petrasii*, *S. haemolyticus*, subsp. *pragensis* and *B. cereus* with IZD 13, 13, 12, and 12 mm, respectively, where standard, Ciprofoxacin (100 $\mu$g/mL) revealed a zone of inhibition of 19 mm against all the tested strains [114]. Green-synthesized *Ricinus communis* seeds gold nanoparticles showed activity against *P. aeruginosa*, *B. cereus*, *K. pneumoniae*, *E. coli*, *Salmonella typhi*, *B. cereus*, and methicillin-resistant *S. aureus* with inhibition zone diameters between 15.5 and 18.5 mm by using agar diffusion method where ampicillin (zone of inhibition range: 15.3–18.33 mm) served as a standard [115]. The MICs of Au NPs biosynthesized using *Jatropha integerrima* were 5.0, 10, 2.5, and 2.5 $\mu$g/mL against *B. subtilis*, *S. aureus*, *E. coli*, and *K. pneumoniae*, respectively [116]. *Opuntia dillenii* extract and its synthesized Au NPs showed inhibition against *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *S. aureus* [117]. However, the Au NPs (20 $\mu$L of 4.0 mM) synthesized using *Capsicum chinense* were found inactive against *S. marcescens E. coli*, *S. aureus*, and *E. faecalis* [118].

Au NPs in this study are primarily spherical (3–100 nm), in addition to hexagonal, cubic crystalline, and triangular (Table 1). Various morphologies have been reported for Au NPs, including spheres, rods, clusters, cubic, icosahedral, decahedral, nanocages, stars, triangular, prisms, hexagonal, and others [52, 73, 99, 103]. The alteration in the ratio of metal salts to apiin compound (derived from the leaves of *Lawsonia inermis* L.) resulted in the modulation of the size and form of the Au and Ag NPs [119]. According to Hussain et al. [120], when Au NPs were synthesized using the chemical reduction process with different solvent polarities, high solvent polarity resulted in spherical Au NPs, as compared to irregular Au NPs with low solvent polarity. The concentration of salts, phytoconstituents, and different parameters of distinct procedures utilized for NPs synthesis might influence the final shape of the NPs. In contrast to certain physicochemical methods, biosynthesis may result in NPs with more clearly defined sizes and morphologies [121].

In the characterization context, FT-IR, TEM, UV–Vis, XRD, and EDAX were the most commonly employed characterization methods (Figure 4(a)). Zeta potential (ZP) was done only in four studies, while the thermal stability of NPs was analyzed in one study. ZP is critical for determining NP stability; a high zeta potential value indicates high stability attributed to a considerable electric charge on a surface [48].

Different types of Au NPs described in the table were promising in efficiency against a wide range of microbial pathogens. *E. ribes* (seeds) and Thyme Au NPs demonstrated potent efficacy against a wide range of bacterial pathogens with the highest zone of inhibition of 34 mm and MIC value of 1.95 $\mu$g/mL, respectively (Table 1). It is assumed that the constituents present in the seeds of *E. ribes*, like alkaloids, quinones, and saponins, may be responsible for the NPs' antibacterial efficacy. Subsequently, the shape, size, solubility, and capability to generate free biocidal metal ions directly influence nanoparticle's antimicrobial efficacy [122]. Smaller NPs generally have higher antibacterial activity than larger ones [123].

Similarly, Table 1 also demonstrated that NPs <10 nm seemed to have more substantial antimicrobial efficacy than particles with >10 nm size. Thus, the size of NPs holds excellent promise in biological applications. The biological potential of plant-mediated NPs depends significantly on the selection of plants and the plant part. The researchers mainly utilize the leaves of the plants (12 studies), followed by whole plants, seeds, and others (Figure 4(b)). Figure 4 provides a comprehensive overview of methods employed to characterize NPs and the plant parts used.

Subsequently, there are few toxicity investigations on Au NPs. Hamelian et al. [98] found no significant toxicity when evaluating the cytotoxicity effect (MTT assay) of Au NPs synthesized using Thyme on HeLa cell lines. The physicochemical characteristics of Au NPs, including their shape, size, surface charge, synthesis method, dose, the route of administration, can affect their toxicity. Several researchers have reported varied toxicity profiles of Au NPs by utilizing different models. To support the safe use of Au NPs in biomedicine, it is necessary to address the contradicting findings about their toxicity [124, 125].

## 5. Mechanism of Action of Green Synthesized NPs: Two-Way Attack

Despite the development of various technologies and formulations over time, the exact mechanism of action of metallic NPs and plant bioactive components against bacteria and fungi remains uncertain. However, several examples in the literature support the idea that the mechanisms of action of metallic NPs in bacteria and fungi are nearly identical. Figure 5 proposes a mechanism for the antimicrobial activity of plant-mediated nanoparticles based on a two-way attack comprising of NPs [126] and plant-bioactive constituents [90]. Recently, Balkrishna et al. [127] provided insights about the two-way attack that needs evidence-based validation in the future.

First, the antimicrobial action of metal NPs includes direct contact with the cell wall/cell membrane, biofilms inhibition, disruption of the cell membrane, alteration of its permeability and metal ion release, generation of ROS, and modulation of signal transduction pathways (Figure 5) [36, 45, 126, 128]. Sharmin et al. [126] reported that NPs may interact with DNA molecules after entering the cell, causing the helical structure to be disrupted by cross-linking inside the nucleic acid strands. Second, the bioactive compounds from the plants attached to the surface of NPs exhibit antimicrobial potential that can be mediated via suppressing efflux pump expression, DNA and protein synthesis, biofilm, and other processes, as demonstrated in Figure 5 [90, 129–131].

Table 1: Characterization and antimicrobial profile of plant-mediated Au NPs.

| Botanical name (part used) | Reaction time (temperature) | Methods used for characterization | Size (morphology) | Targeted microbes | Major findings | Standard (ZI) | Data source |
|---|---|---|---|---|---|---|---|
| *Actinidia deliciosa* (fruit) | NM (30, 60, and 80°C) | EDAX, XPS, TEM, SAED, XRD, FTIR, ZP | 20 nm (spherical) | PA | Active (10–30 $\mu$L) | NM | [91] |
| *Justicia glauca* (leaves) | 10 min (RT) | UV–Vis, FTIR TEM, XRD, EDAX | 32.5 nm (hexagonal and spherical) | ML, BS, SA, SM, LA, EC, PA, SC, CA | ZI: 9–17 mm (100 $\mu$g) MIC: 6.25–25 $\mu$g/mL | Azithromycin (ZI:17–24 mm at 100 $\mu$g) Clarithromycin (ZI: 16–24 mm at 100 $\mu$g) | [92] |
| *Rhazya stricta* (herb) | 60 min (24°C) | UV–Vis, XRD, TEM, FTIR, EDX, HRTEM, DLS, ZP | 40 nm (cubic crystalline) | EC, SA | MIC: 25 and 50 $\mu$g/mL | Streptomycin sulfate (NM) | [93] |
| *Embelia ribes* (seeds) | NM | UV–Vis, DLS, HR-TEM, FTIR, XRD | 10–30 nm (spherical) | EC, SA | ZI: 34 and 27 mm (1,000 $\mu$g/mL) | Tetracycline (NM at 30 $\mu$g/mL) | [94] |
| *Indigofera tinctoria* (leaves) | 0.5 min (RT) | UV–Vis, FTIR, XRD, TEM, EDX, AFM | 6–29 nm (spherical) | BP, SA, PS, EC, AFY, ANI | ZI: 10–30 mm (12.5–200 $\mu$g/mL) | NM | [95] |
| *Stereospermum suaveolens* (root bark) | NM | UV–Vis, FTIR, SAED, TEM, XRD, AFM | 27.19 nm (spherical) | BS, SA, EC, PA, ASN, AFL | ZI: 10–25 mm (1 mg/mL) | NM | [96] |
| *Origanum vulgare* (aerial parts) | 1 min (85°C) | TEM, UV–Vis, PCS, FTIR, SERS | 40 nm (spherical) | SA, LM, SED, EC, CA | ZI: 8–28 mm (NM) | NM | [97] |
| *Thymus vulgaris* (plant) | 30 min (RT) | FTIR, XRD, EDS, SEM, AFM, TEM, TGA | 35–40 nm (spherical) | EC, PA, SA, BS | ZI: 22–25 mm (31 $\mu$g/mL) MIC: 1.95–15.62 $\mu$g/mL | Kanamycin and cephalexin (ZI: >20–<30 mm at NM) | [98] |
| *Alpinia nigra* (leaves) | 15 min (95°C) | FTIR, TEM, SEM, UV–Vis, XRD, EDX | 21.52–56.40 nm (hexagonal and triangular) | BS, EC, CA | ZI: 15–18 mm (400 $\mu$g/mL) MIC: 250–350 $\mu$g/mL | Chloramphenicol (ZI: 28–30 mm at 30 $\mu$g) Nystatin (ZI: 30 mm at 50 $\mu$g) | [99] |
| *Nigella arvensis* (leaves) | 20 min (RT) | UV–Vis, XRD, FTIR, TEM | 3–37 nm (spherical) | SE, BS, SA, EC, SM, PA | MIC: 62.5–250 $\mu$g/mL | NM | [100] |
| *Coleus forskohlii* (root) | NM | UV–Vis, HR-TEM, PSA, FTIR, XRD | 10–30 nm (spherical) | PV, ML | ZI: 11–19 mm (250–1,000 $\mu$g/mL) | Tetracycline (ZI: 15 and 19 mm at 30 $\mu$g/mL) | [101] |
| *Amomum villosum* (fruits) | 0.05 min (RT) | UV–Vis, FE-TEM, EDX, XRD, SAED, DLS, FTIR | 5–10 nm (spherical) | SA, EC | ZI: 17.66–18 mm (45 $\mu$g/disc) | Neomycin (ZI: 15–15.33 mm at 30 $\mu$g/disc) | [102] |
| *Bauhinia purpurea* (leaves) | 0.5 min (NM) | UV–Vis, FTIR, XRD, TEM, EDX | NM (hexagonal) | SA, BS, EC, PA, AFL, ASN | ZI: 8–12 mm (50 $\mu$L) | NM | [103] |
| *Garcinia mangostana* (rind of fruit) | NM (30–35°C) | UV–Vis, HR-TEM, SAED, XRD, FTIR, HR-SEM | 20–40 nm (spherical) | PS | ZI: 11 mm (NM) | Vancomycin (ZI: 25 mm at 30 $\mu$g) | [104] |
| *Peganum harmala* (leaves and seed) | 25 min (RT) | UV–Vis, FTIR, FESEM, XRD, EDX, TEM | 43.44–52.04 nm (spherical) | EC, SA | Leaves: ZI: 25–30 mm (200 $\mu$g/mL) Seed: no activity | Cefoxitin (ZI: 25 mm at 150 $\mu$L) Chloramphenicol (ZI: 25 mm at 150 $\mu$L) | [105] |
| *Chenopodium formosanum* (shell) | 1 min (25°C) | EDS, SAED, XRD, HR-TEM, FTIR | 8 nm (spherical) | EC, SA | 59.23%–84.44% (90 $\mu$g/mL) | Streptomycin (98.45%–99.83% at 40 $\mu$g/mL) | [106] |
| *Dillenia indica* (leaves) | 60 min (DNM) | XRD; FTIR; TEM; SAED; UV–Vis | 5–50 nm (spherical) | SA, EC | ZI: 23.5–28 mm (1 and 1.5 mg/mL) | Ciprofloxacin (ZI: 20–23 mm at NM) | [107] |
| *Musa acuminata* (flowers) | NM | UV–Vis, FTIR, XRD, SEM, EDAX | 10.1–15.6 nm (poly-dispersed spherical) | SA, EF, EC, ST, PA, PM, KP | ZI: 7–11 mm (1,000 µg) | Streptomycin (ZI: 13–22 mm at 10 $\mu$g) | [108] |
| *Coleus aromaticus* (leaves) | 10 min (NM) | UV–Vis, XRD, FTIR, SEM, HR-TEM, DLS, SPR, UV-DRS | 16–18 nm (spherical) | SE, EC | ZI: 22 and 27 mm (NM) | NM | [109] |

Table 1: Continued.

| Botanical name (part used) | Reaction time (temperature) | Methods used for characterization | Size (morphology) | Targeted microbes | Major findings | Standard (ZI) | Data source |
|---|---|---|---|---|---|---|---|
| *Clerodendrum inerme* (leaves) | 65 min (80°C) | TEM, XRD, FTIR, UV–Vis, DLS | 5.82 nm (spherical) | BS, SA, K, EC, ANI, AFL, TH | ZI: 13–25 mm (125–1,000 $\mu$g/mL) | Cephradine (ZI: <12–>16 mm at NM) Terbinafine hydrochloride (ZI: >15–<24 mm at NM) | [110] |
| *Mangifera indica* (seed) | 5 min (NM) | UV–Vis, XRD, TEM, SAED, FTIR, ZP | 9.45 nm (round) | BC, BS, SA, CR, EC, PA, KP, STM, CN, CG, FCS | ZI: 5–10 mm (22 mg/mL) | NM | [111] |
| *Solanum nigrum* (leaves) | 10 min (NM) | TEM, XRD, EDAX, UV-Vis, FTIR | 9.39 nm (spherical) | EC | ZI: 19.2–22 mm (10 $\mu$g/mL) | NM | [112] |
| *Acalypha indica* (leaves) | 5 min (NM) | UV–Vis, XRD, FTIR, SEM, HR-TEM | 20 nm (spherical) | SE, EC | ZI: 31 and 26 mm | NM | [113] |
| *Cynodon dactylon* (plant) | NM | FT-IR, XRD, SEM, TEM | 22–34 nm (spherical) | ENC, SPP, STH, BC | ZI: 12–13 mm (100 $\mu$g/mL) | Ciprofloxacin (ZI: 19 mm at 100 $\mu$g/mL) | [114] |
| *Ricinus communis* (seeds) | 1–24 hr (30–80°C) | UV–Vis, TEM, FTIR | 100 nm (spherical) | EC, PA, BC, KP, ST, MRSA | ZI: 15.5–18.5 mm (100 $\mu$L) | Ampicillin (ZI: 15.33–18.33 mm) | [115] |
| *Jatropha integerrima* (flowers) | NM | UV–Vis, TEM, FTIR, EDX, SAED, XRD, ZP | 38.8 nm (spherical) | SA, BS, EC, KP | MIC: 2.5–10 $\mu$g/mL | Ampicillin (ZI: 14.33–21.33 mm at 10 $\mu$g/mL) | [116] |
| *Opuntia dillenii* (whole plant) | 5 hr (RT) | UV–Vis, SEM, FTIR, EDX | 45–77 nm (roughly spherical) | BS, SA, EC, PA, ST | ZI: 13.21–17.2 mm (NM) | Amoxicillin (ZI: 23.65–29.66 mm at NM) | [117] |
| *Capsicum chinense* (leaves) | NM | UV–Vis, FTIR-ATR, XPS, UHR-SEM-EDX, TEM | 15–17 nm (spherical) | SA, EC, EF, SM | Not active | Kanamycin (ZI: 21.39 mm) | [118] |

Note: Not mentioned (NM); Room temperature (RT); Zeta potential (ZP); X-ray photoelectron spectroscopy (XPS); X-ray powder diffraction (XRD); UV–visible spectrophotometry (UV–Vis); Field-emission transmission electron microscopy (FE-TEM); Transmission electron microscopy (TEM); Particle size analysis (PSA); Thermogravimetric analysis (TGA); Dynamic light scattering (DLS); Elemental mapping (EP); Selected area electron diffraction (SAED); Field-emission scanning electron microscopy (FESEM); Energy-dispersive X-ray spectroscopy (EDS); Surface plasmon resonance (SPR); UV-diffuse reflectance spectroscopy (UV-DRS); Surface-enhanced Raman spectroscopy (SERS); Fourier-transform infrared spectroscopy (FT-IR); Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR); Scanning electron microscopy (SEM); Ultra high-resolution scanning electron microscopy coupled to energy-dispersive X-ray spectroscopy (UHR-SEM-EDX); High-resolution transmission electron microscopy (HR-TEM); Energy dispersive X-ray analysis (EDX or EDAX); Photon correlation spectroscopy (PCS); Atomic force microscopy (AFM); *Pseudomonas aeruginosa* (PA); *Micrococcus luteus* (ML); *Staphylococcus aureus* (SA); *Bacillus subtilis* (BS); methylene resistant *Staphylococcus aureus* (SA); *Lactobacillus acidophilus* (LA); *Streptococcus mutans* (SM); *Candida albicans* (CA); *Bacillus pumilus* (BP); *Pseudomonas* sp. (PS); *Saccharomyces cerevisiae* (SC); *Escherichia coli* (EC); *Aspergillus fumigatus* (AFU); *Aspergillus niger* (ANI); *Aspergillus nidulans* (ASN); *Aspergillus flavus* (AFL); *Listeria monocytogenes* (LM); *Salmonella enteritidis* (SED); *Staphylococcus epidermidis* (SE); *Serratia marcescens* (SM); *Salmonella enterica* (SEC); *Proteus vulgaris* (PV); *Micrococcus luteus* (ML); *Enterococcus faecalis* (EF); *Salmonella typhi* (ST); *Proteus mirabilis* (PM); *Klebsiella pneumoniae* (KP); *Staphylococcus epidermidis* (SE); *Klebsiella* sp. (K); *Klebsiella pneumoniae* (KP); *Trichoderma harzianum* (TH); *Bacillus cereus* (BC); *Candida glabrata* (CG); *Corynebacterium rubrum* (CR); *Salmonella typhimurium* (STM); *Cryptococcus neoformans* (CN); fungal clinical isolates No.12, 15, 22 (FCS); *Enterobacter cloacae* (ENC); *Staphylococcus haemolyticus* (STH); *Staphylococcus petrasii* subsp. *pragensis* (SPP); Zone of inhibition (ZI); Minimum inhibitory concentrations (MIC).

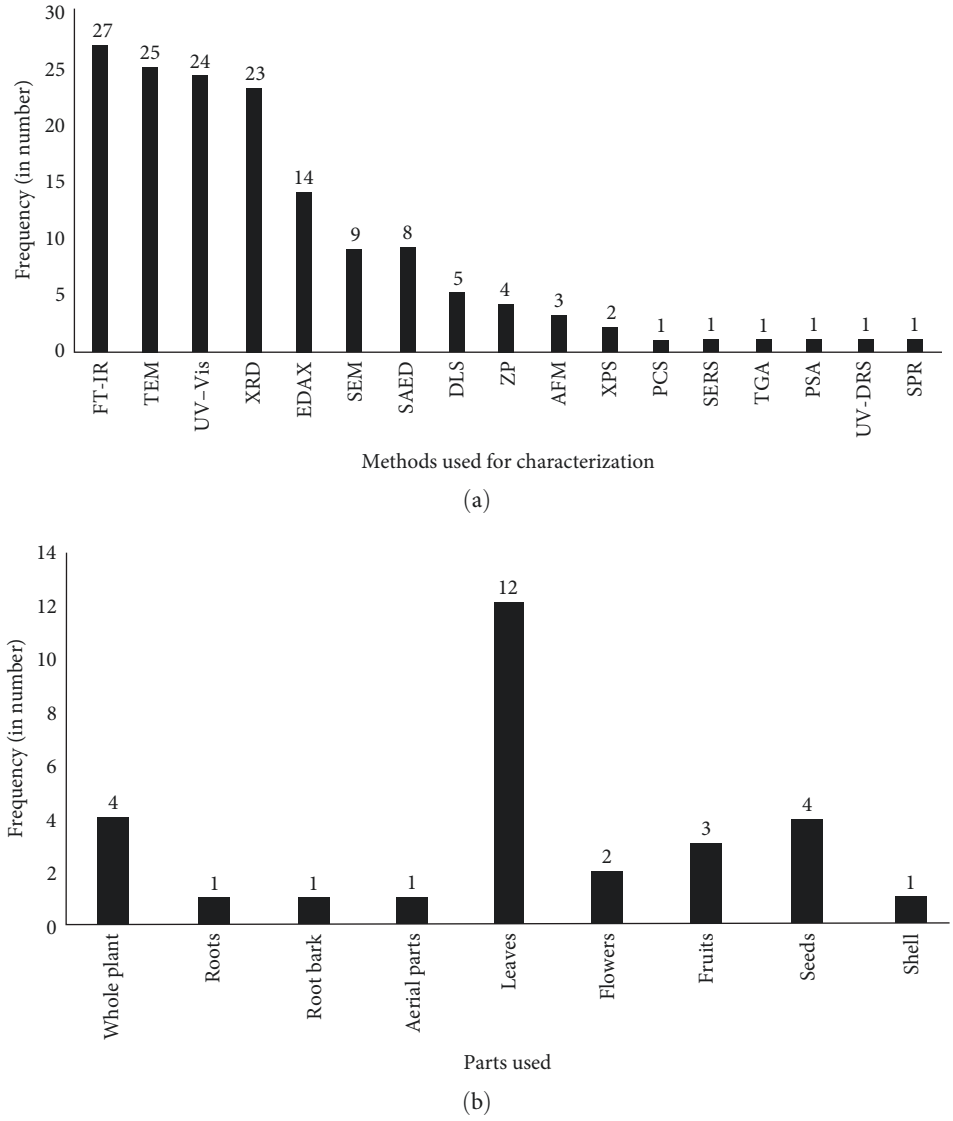


Figure 4: Frequency of methods used for NPs characterization (a), and plant part used for green synthesis of NPs (b).

## 6. Commercialization of Au NPs

Drug resistance among bacterial strains is a significant problem that can be effectively solved by synthesizing antibacterial NPs. Because they are good drug carriers with outstanding biocompatibility and bactericidal capability, Au NPs can increase the antibacterial effects of loaded antibacterial medications. The green synthesized Au NPs are one of them and have been effectively tested against various pathogenic and drug-resistant bacterial strains. They have numerous biological and therapeutic applications. Additionally, their use as a means of drug delivery in managing tumors has been noted [132, 133]. The role of Au NPs in protecting nucleic acids from being degraded by nucleases was reported by Klębowski et al. [134]. By covering Au NPs with a biopolymer that can firmly adsorb insulin to its surface, Joshi et al. [135] employed Au NPs as protein carriers. They validated the increased effectiveness of insulin delivery. The coupled Au NPs with oligonucleotides and their special qualities, according to Hu et al. [136], have allowed them to be used as prospective gene carriers. In addition, the function of Au NPs in therapies such as photothermal therapy, photodynamic therapy, and radiation therapy has also been noted [136, 137]. The literature has also documented Au NPs' antioxidant and photocatalytic activity. Commercial Au NPs can be used in diverse biomedical applications. More study on biosynthetic procedures with well-defined size and shape is needed before Au NPs are widely used in commercial applications.

## 7. Conclusion and Future Perspectives

In conclusion, different researchers have successfully achieved the green synthesis of Au NPs (3–100 nm) using diverse plant extracts. All plant parts have been utilized, leaves being the most commonly employed part. Most of the studies only revealed a zone of inhibition, which is a major gap in

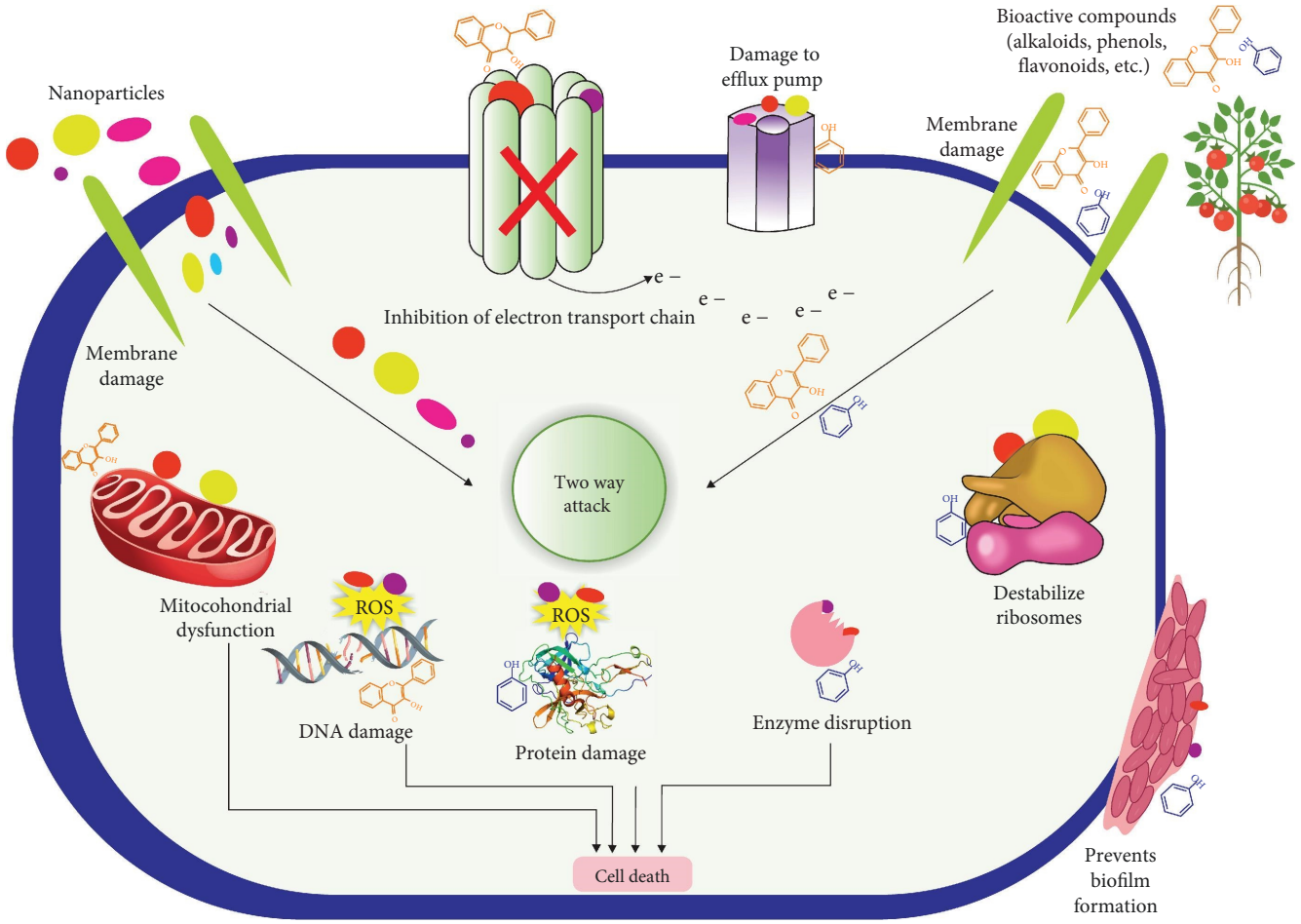


Figure 5: Mechanistic insights into the antimicrobial effect of green synthesized nanoparticles.

antimicrobial studies; MIC and MBC were performed in a few studies. The antimicrobial studies should be supplemented with toxicity studies to maximize the benefit of these studies as it is early to comment on the toxicity of NPs as most of the studies are in preliminary stages. Notably, the stability of NPs cannot be ignored. The process for NPs synthesis must be optimized as the biological activity of NPs is influenced by various factors, including size, shape, and reaction variables. Nanotechnology-based formulations could be used in the fight against drug-resistant pathogens and to prevent the never-ending resistance pandemic. Intriguingly, the ability to modify the properties of Au NPs by merely altering their size or form will be used in novel applications in the future. Docking programs for deducing the mechanisms of NPs and phytoactive components will aid in broadening the field of smart antimicrobials that could be utilized to combat drug resistance. Furthermore, drug design theoretical frameworks can be used to suggest the possibility of green synthesized NPs as novel drugs. As biosynthesis of NPs has developed as a prominent field of nanobiology, there are numerous potentials for investigating innovative green preparatory approaches.

## Data Availability

All data are included in the manuscript.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

A. B. supervised the manuscript. A. K. (Ashwani Kumar), A. R., N. T. (Nikesh Thakur), and V. A. wrote the first draft. S. M. and V. K. contributed in figures and in providing literature. N. T. (Naveen Thakur), A. K. (Amita Kumari), and N. K. critically reviewed the first draft. A. K. (Ashwani Kumar) and V. A. improved the first draft. The final submitted version of the manuscript has been seen and approved by all contributors.

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