



Review

Green Synthesis, Characterization, and Potential Antibacterial and Anticancer Applications of Gold Nanoparticles: Current Status and Future Prospects

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Abstract: Drug resistance is a serious problem for human health worldwide. Day by day this drug resistance is increasing and creating an anxious situation for the treatment of both cancer and infectious diseases caused by pathogenic microorganisms. Researchers are trying to solve this terrible situation to overcome drug resistance. Biosynthesized gold nanoparticles (AuNPs) could be a promising agent for controlling drug-resistant pathogenic microorganisms and cancer cells. AuNPs can be synthesized via chemical and physical approaches, carrying many threats to the ecosystem. Green synthesis of AuNPs using biological agents such as plants and microbes is the most fascinating and attractive alternative to physicochemical synthesis as it offers many advantages, such as simplicity, non-toxicity, cost-effectiveness, and eco-friendliness. Plant extracts contain numerous biomolecules, and microorganisms produce various metabolites that act as reducing, capping, and stabilizing agents during the synthesis of AuNPs. The characterization of green-synthesized AuNPs has been conducted using multiple instruments including UV–Vis spectrophotometry (UV–Vis), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray diffraction (XRD), DLS, and Fourier transform infrared spectroscopy (FT-IR). AuNPs have detrimental effects on bacterial and cancer cells via the disruption of cell membranes, fragmentation of DNA, production of reactive oxygen species, and impairment of metabolism. The biocompatibility and biosafety of synthesized AuNPs must be investigated using a proper in vitro and in vivo screening model system. In this review, we have emphasized the green, facile, and eco-friendly synthesis of AuNPs using plants and microorganisms and their potential antimicrobial and anticancer applications and highlighted their antibacterial and anticancer mechanisms. This study demonstrates that green-synthesized AuNPs may potentially be used to control pathogenic bacteria as well as cancer cells.



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1. Introduction

Drug resistance has become a global challenge for human health, leading to increased morbidity, mortality, and economic burden. In the realm of cutting-edge scientific advancements, the synthesis and utilization of nanomaterials have emerged as a revolutionary avenue with profound implications for medical and healthcare applications [1]. Nanoparticles are small particles and have significant applications in the healthcare and environmental sectors. They play an important role in drug development, drug delivery, tissue engineering, wound healing, water treatment, soil remediation, air pollution control, etc. [2–4]. Nanoparticles can be synthesized via chemical and physical approaches, carrying many threats to the ecosystem. The main drawbacks of physicochemical methods of nanoparticle synthesis are the potential use of hazardous chemicals, generation of hazardous byproducts, high energy consumption, and high cost. The green synthesis of nanoparticles using biological agents such as plants and microbes is the most fascinating and attractive alternative to physicochemical synthesis as it offers many advantages such as simplicity, non-toxicity, cost-effectiveness, and eco-friendliness. Plant extracts contain numerous biomolecules, such as alkaloids, flavonoids, terpenoids, phenolic compounds, etc., that act as reducing, capping, and stabilizing agents during the synthesis of nanoparticles. Similarly, microorganisms produce various primary and secondary metabolites, such as amino acids, enzymes, flavonoids, etc., that serve as reducing, capping, and stabilizing agents for the synthesis of nanoparticles. These plants and microbe-derived biomolecules facilitate the reduction of gold ions into gold nanoparticles (AuNPs), prevent aggregation, and stabilize the final nanoparticles. Notably, the green synthesis of nanoparticles has garnered significant attention due to its eco-friendly and sustainable approach. Nanoparticles synthesized using natural compounds derived from plants, bacteria, or fungi, hold immense promise in various fields, notably in the domains of anticancer and antimicrobial treatments [4–8].

The convergence of nanotechnology and green synthesis has paved the way for a new era of biomedical research and development [9,10]. In the fight against cancer, conventional treatments often come with severe side effects and limitations. However, the advent of green-synthesized nanoparticles offers a potential solution. These nanoparticles, synthesized using plant extracts rich in bioactive compounds, exhibit remarkable anticancer properties [11,12]. By targeting cancer cells with enhanced precision and minimizing damage to healthy cells, these nanoparticles hold the potential to revolutionize cancer therapy, ushering in a new era of more effective and less toxic treatments [7,13]. At the heart of this technological leap lies the remarkable world of nanoparticles. These tiny structures, typically ranging from 1 to 100 nanometers, can be engineered to possess unique physical, chemical, and biological properties due to their quantum-size effects and high surface-to-volume ratios [14,15]. Such attributes have granted nanoparticles the ability to interact with biological systems in profound ways, offering a wide range of medical applications [5,16,17]. While the potential of nanoparticles is unquestionable, concerns regarding the environmental impact of their synthesis have prompted researchers to explore sustainable alternatives. Green synthesis of nanoparticles has emerged as a promising solution, capitalizing on the inherent properties of natural compounds to reduce the need for harsh chemicals and energy-intensive processes [7,18,19]. This approach aligns with

the principles of green chemistry and provides a pathway to environmentally friendly nanoparticle production [20,21].

The battle against cancer is a major global challenge, driving relentless efforts to discover novel, effective, targeted therapies [21,22]. Green-synthesized nanoparticles have emerged as a potential game-changer in this arena. The natural compounds in their synthesis often contain bioactive agents with inherent anticancer properties. When harnessed to fabricate nanoparticles, these compounds can be directed to selectively target cancer cells while minimizing harm to healthy tissues, offering a more focused and less toxic alternative to conventional treatments [23]. Nanoparticles have detrimental effects on cancer cells via the disruption of cell membranes, fragmentation of DNA, production of reactive oxygen species, and impairment of metabolism. The unique physicochemical properties of nanoparticles enable them to penetrate cell membranes, deliver therapeutic payloads, and induce controlled cell death, thereby enhancing the efficacy of anticancer treatments [24]. The growing menace of antimicrobial resistance has underscored the urgency for innovative strategies to combat infectious diseases. Green-synthesized nanoparticles have emerged as a potential tool in the fight against drug-resistant microbes. Their small size and high surface area provide ample opportunities for interaction with microbial cells, disrupting their membranes, interfering with essential cellular processes, and impeding their proliferation [25,26]. This multi-pronged approach offers a distinct advantage, as it reduces the likelihood of developing resistance to nanoparticle-based antimicrobial agents [27]. By harnessing the power of nature's compounds, these nanoparticles hold the potential to overcome the limitations of traditional antibiotics and contribute to the battle against infectious diseases [28,29].

Beyond their scientific significance, the economic implications of green-synthesized nanoparticles in anticancer and antimicrobial applications are considerable [30]. As research in this field advances, it has the potential to stimulate various industries, from pharmaceuticals to biotechnology, creating opportunities for innovation, job creation, and economic growth [31]. Developing novel therapies can reduce healthcare costs, as more targeted and effective treatments mitigate the need for lengthy hospital stays and extensive follow-up care [32]. Furthermore, adopting green synthesis aligns with sustainable practices, enhancing corporate social responsibility profiles and appealing to environmentally conscious consumers and investors. Simultaneously, the rise of antibiotic-resistant microbes has fueled the urgent need for novel antimicrobial agents. Green-synthesized nanoparticles, owing to their unique physicochemical properties and diverse biological activities, are emerging as promising candidates for combating microbial infections. The antimicrobial efficacy of these nanoparticles stems from their ability to disrupt microbial cell membranes, inhibit vital enzyme systems, and interfere with microbial replication, thereby offering a multifaceted approach to addressing the growing antimicrobial resistance crisis [20,33].

As we delve deeper into green-synthesized nanoparticles, it becomes increasingly evident that their potential extends far beyond conventional therapeutic approaches. The amalgamation of eco-friendly synthesis methods with nanoparticle technology offers a sustainable path forward, harnessing the power of nature to tackle some of the most pressing challenges in modern medicine. This exploration into the anticancer and antimicrobial applications of green-synthesized nanoparticles promises to reshape the medical landscape, offering hope for more effective, efficient, and environmentally conscious treatment strategies. Among various metal nanoparticles, AuNPs have received a lot of attention due to their wide scope of application in different branches of biomedical science [5,7,34,35]. Many reports suggest applying AuNPs as antibacterial and anticancer agents to control drug-resistant pathogenic microorganisms and cancer cells, respectively [5,7,12,36]. Because of their strong antimicrobial and anticancer efficacies, there is prominent research interest

in AuNPs for developing novel, safe, and effective antimicrobial and anticancer agents. Figure 1 shows the schematic representation of the green synthesis, characterization and potential antibacterial and anticancer applications of AuNPs.

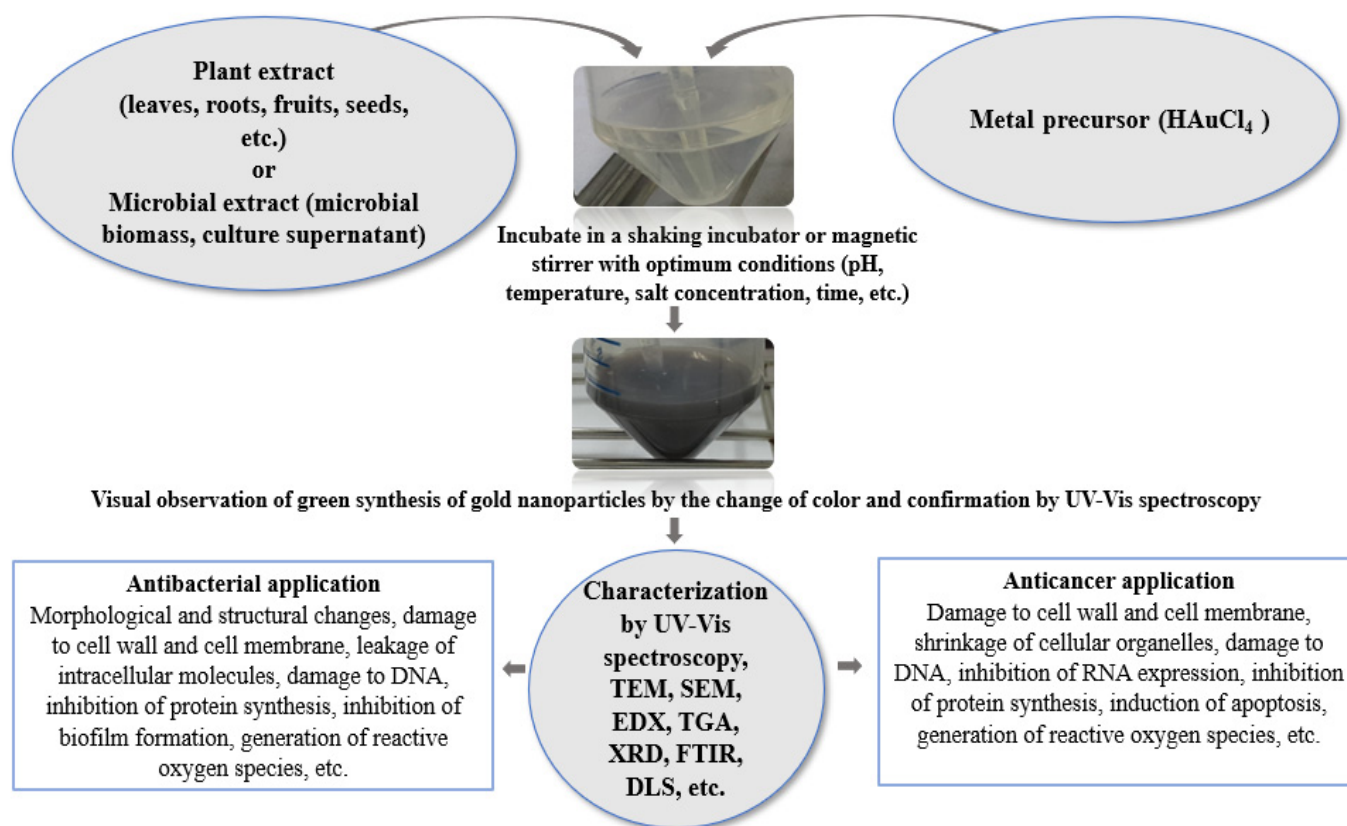


Figure 1. Schematic representation of the green synthesis, characterization and potential antibacterial and anticancer applications of AuNPs.

This review article provides an overview of the green synthesis of AuNPs using plants and microorganisms and their potential applications against drug-resistant pathogenic bacteria and cancer cells. This review also focuses on the antibacterial and anticancer mechanisms of facile and eco-friendly synthesized AuNPs.

2. Plant-Mediated Green Synthesis

The availability of many different plants and their simple and secure use make plant-mediated synthesis of AuNPs an extensively used approach [37]. For green synthesis of bioactive AuNPs, a variety of plant materials, e.g., fruits, roots, flowers, leaves, peels, etc., have been used successfully (Table 1). Many bioactive substances, e.g., proteins, enzymes, amino acids, terpenoids, tannins, saccharides, phenols, and flavonoids, are found in plant extracts [38]. Plant extracts are stable and efficient in the production of bioactive AuNPs [39]. In the last few years, many studies have been performed for the green synthesis of bioactive AuNPs by utilizing different parts of plants, e.g., leaf, bark, rhizome, seed, fruit, seaweed, latex, flower, peel, and stem, as well as whole plant (Table 1). For example, Sundararajan and Kumari [40] utilized leaf extracts from *Artemisia vulgaris* for rapid and eco-friendly synthesis of bioactive AuNPs. They also examined the antifungal and antimicrobial activity of biosynthesized AuNPs against various pathogenic fungi and bacteria. Nadagouda et al. [41] synthesized AuNPs by utilizing rhizome extracts from *Curcuma longa*. Fruit extract of *Citrus maxima*, citrus (lemon, tangerine, and orange), and *Genipa americana* L., *Lantana camara* were utilized by Majumdar et al. [42], Sujitha and

Kannan [43], Sengupta et al. [44], Yu et al. [45], Kumar et al. [46], Dipankar et al. [47], Hidayat et al. [48], and Kumar et al. [49] for the eco-friendly synthesis of AuNPs. The seeds and pulp of *Abelmoschus esculentus*, *Caesalpinia crista*, *Linum usitatissimum*, *Murraya koenigii*, and *Theobroma cacao* were utilized for the green synthesis of AuNPs by Rahaman Mollick et al. [50], Jayaseelan et al. [51], Donga et al. [52], Al-Radadi et al. [53], Ananth et al. [54], and Dwivedi et al. [55]. Peel extracts of different plants, for example *Mangifera indica* Linn (Mango), *Musa paradisiaca*, and *Punica granatum*, have also been investigated for the synthesis of bioactive AuNPs (Table 1).

Optimum synthesis conditions (salt concentration, temperature, and incubation time), size (nm), shape of synthesized AuNPs, and their bioactivity vary greatly depending on the plant or part of the plant used. According to Philip [56], AuNPs of 17 to 20 nm in size were synthesized using the leaf extract of *Mangifera indica* in a two minute reaction. On the other hand, according to Yang et al. [57] AuNPs of 3.26 to 21.68 nm were synthesized using peel extract of *Mangifera indica* Linn (Mango) within 15 mins of reaction. According to Barai et al. [58] stem/bark extracts of *Nerium oleander* produced spherical, hexagonal, triangular, and rod-shaped AuNPs. The leaf extracts of *Abutilon indicum*, *Artocarpus hirsutus*, *Curcuma Kwangsiensis*, *Dracocephalum kotschyi*, *Hibiscus sabdariffa*, *Mangifera indica*, *Mimosa pudica*, *Platycodon grandiflorum*, *Ricinus ommunis*, and *Terminalia arjuna* produced spherical-shaped AuNPs (Table 1). Various physicochemical parameters, such as type of plant, composition of extract, salt concentration, plant extract concentration, extract salt ratio, incubation time, incubation temperature, pH, etc. have a significant impact on the yield, shape, size and stability of the synthesized AuNPs [59–62]. These physicochemical parameters also influence the efficacy of synthesized AuNPs. For example, increasing the volume of *Carallia brachiata* leaf extract decreased the size of the synthesized AuNPs [61]. Similarly, by increasing the concentration of HAuCl₄ in the reaction mixture, the synthesis rate, size and shape of *Solidago canadensis* leaf extract mediated AuNPs changed dynamically [62]. According to Singh et al. [63], the size of *Mangifera* peel extract-mediated AuNPs was 6 nm in pH 9 and 18 nm in pH 2. Similarly, the size of *Padina tetrastromatica*-mediated AuNPs was changed with different temperatures [64]. Diksha et al. [65] investigated the optimum conditions for the green synthesis of AuNPs using leaf extract of *Syzygium cumini* and they found that reaction parameters such as salt concentration, plant extract concentration, pH, temperature, and time play an important role in the facile, stable, and rapid synthesis of bioactive AuNPs. Plant-mediated production of AuNPs is likely mediated by the chemistry of reduction and oxidation. According to certain theories, plant extracts include vitamins, proteins, amino acids, organic acids, enzymes, terpenoids, flavonoids, alkaloids, polyphenols, and polysaccharides, which play important roles in the reduction of gold salts as well as acting as capping and stabilizing agents [66,67].

Table 1. Plant-mediated green synthesis and potential antibacterial and anticancer applications of AuNPs.

Plant	Used Part	Optimum Synthesis Conditions (Salt Concentration, Temperature, Incubation Time)	Size (nm)	Shape	Applications	Reference
<i>Clerodendrum trichotomum</i>	Leaf extract	10.20 g HAuCl ₄ was added to 100 mL of plant extract, incubated at 65 °C, 80 min	19.1 ± 2.2 (Average)	Spherical	Antibacterial application against <i>Klebsiella pneumoniae</i> and <i>Staphylococcus aureus</i> . Anticancer application against breast cancer cell line (MCF-7)	[2]

Table 1. Cont.

Plant	Used Part	Optimum Synthesis Conditions (Salt Concentration, Temperature, Incubation Time)	Size (nm)	Shape	Applications	Reference
Henna	Leaf extract	10 mM HAuCl ₄ , room temperature, 30 min	6 ± 2.5 (Average)	Spherical	Antibacterial activity against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	[68]
<i>Halodule uninervis</i>	Leaf extract	HAuCl ₄ ·3H ₂ O, 70–80 °C, 30 min	10–50	Spherical	Anticancer activity against human breast cancer cells MDA-MB-231	[69]
Tangerine	Peel extract	15 mM HAuCl ₄ ·3H ₂ O, 40 °C, 60 min	26 ± 5 (Average)	Spherical	Antibacterial activity against <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , and <i>Pseudomonas aeruginosa</i>	[70]
<i>Aconitum violaceum</i>	Plant extract	1 mM HAuCl ₄ , room temperature, 50 min	<100 (Average)	Spherical and triangular	Antibacterial activity against <i>Lactobacillus acidophilus</i> and <i>Escherichia coli</i>	[71]
<i>Syzygium cumini</i>	Leaf extract	HAuCl ₄ ·3H ₂ O, ambient temperature, 24 h	120.5 (Average)	Spherical	Antibacterial application against <i>Aeromonas hydrophila</i> , <i>E. coli</i> , <i>Salmonella Typhimurium</i> , <i>P. aeruginosa</i> , <i>Enterococcus faecium</i> , <i>Pediococcus</i> sp., and <i>Bacillus cereus</i>	[72]
<i>Zingiber officinale</i> , curcumin	Root extract	HAuCl ₄ solution (0.1 mM) and ginger extract mixture is kept boiling and stirring at 600 rpm until the solution turned purple	20 (Average size)	Spherical and oval	Antimicrobial efficacy against, <i>E. coli</i> , <i>P. aeruginosa</i> and <i>S. aureus</i>	[73]
<i>Abutilon indicum</i>	Leaf extract	1 mM HAuCl ₄ , room temperature, 2 min	10–20	Spherical	Effective against human colon cancer	[12,74]
<i>Artemisia vulgaris</i>	Leaf extracts	1 mM HAuCl ₄ solution, room temperature, 24 h	50–100	Spherical, triangular, and hexagonal	Antibacterial application against <i>S. aureus</i> , <i>S. pyogenes</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , anti-fungal activity against <i>Aspergillus niger</i> , induced apoptosis in MCF-7 (breast cancer)	[36,40]
<i>Azadirachta indica</i>	Leaf extract	100 ppm gold chlorate, the extract and the gold chlorate mixture are boiled till the appearance of wine-red color.	≤121.7	Spherical, hexagonal, and triangular	Anticancer activity on HeLa and MDCK cell	[75]
<i>Areca catechu</i>	Nut	30 mL chloroauric acid, 10 mL aqueous nut extract, 4–5 h	22.2	Spherical	Anticancer activity on HeLa	[76]
<i>Acacia nilotica</i>	Bark extract	1 mM HAuCl ₄ , room temperature, 10 min	10–15	Unshaped, quasispherical	Anticancer activity on hepatic cell, antibacterial activity against <i>B. subtilis</i> and <i>S. aureus</i>	[77]
<i>Acorus calamus</i>	Rhizome extract	2.5 mL of extract, 2.5 mL of 0.001 M chloroauric acid, stir at 240 rpm until the color turns dark brown.	10	Spherical	Antibacterial activity <i>S. aureus</i> and <i>E. coli</i>	[33,78]
<i>Artocarpus hirsutus</i>	Leaf extract	1 mM HAuCl ₄ , at 80 °C, about 12 h.	5–40	Spherical	Efficacy against human cancer cell lines (HeLa, RKO and A549).	[34,79]

Table 1. Cont.

Plant	Used Part	Optimum Synthesis Conditions (Salt Concentration, Temperature, Incubation Time)	Size (nm)	Shape	Applications	Reference
<i>Abelmoschus esculentus</i>	Seed and pulp extract	(1 mM) $\text{HAuCl}_4 \times \text{H}_2\text{O}$ (95 mL) at room temperature, 1 h	45–75	Spherical, uneven shape	Antibacterial activity against <i>E. coli</i> , <i>P. aeruginosa</i> , <i>B. cereus</i> , and <i>B. subtilis</i> . Antifungal activity against <i>A. niger</i> , <i>Puccinia graminis tritici</i> , and <i>C. albicans</i>	[50,51]
<i>Butea monosperma</i>	Leaf extract	0.01 M of HAuCl_4 , room temperature, 35 min	20–80	Mainly spherical but with a few rods; irregular and hexagonal	Anticancer activity on B16F10, MCF-7, HNGC2, A549, HUVEC and ECV-304	[12,80]
<i>Cassia auriculata</i>	Leaf extract	1 mM auric chloride solution, room temperature, 10 min.	15–25	Spherical, triangular, and hexagonal	Antibacterial efficacy against <i>Bacillus subtilis</i> , <i>K. pneumonia</i> , <i>P. aeruginosa</i>	[81]
<i>Caesalpinia crista</i>	Seed extract	1 mM HAuCl_4 , room temperature ($25^\circ\text{C} \pm 2^\circ\text{C}$) for 24 h	15.13	Spherical	Antibacterial efficacy against <i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> . Anticancer activity against human cancer cell lines (HeLa, MCF-7),	[52]
Citrus (lemon, tangerine, orange)	Fruit extract	1 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, room temperature	32.3, 43.4, 56.7	Spherical and triangular	Anticancer effect on the growth of HepG2 (liver cancer cell line)	[42,43]
<i>Citrus maxima</i>	Fruit extract	(1%, w/v) $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$, room temperature, 5 min.	15–35	Spherical	Antibacterial efficacy against <i>Staphylococcus aureus</i>	[44,45]
<i>Clitoria ternatea</i>	Leaf extract	99 mL of 10^{-3} aqueous HAuCl_4 , room temperature for (0 min–24 h)	100	Rod	Antibacterial activity against <i>E. coli</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> , and <i>S. pyogenes</i>	[82,83]
<i>Curcuma longa</i>	Rhizome extract	0.01 mL HAuCl_4 , room temperature, overnight culture	5–60	Oblong and spherical	Anticancer effect on the lung cancer cells	[41]
<i>Curcuma Kwangsiensis</i>	Leaf extracts	(1 mM) $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$, 25°C , 1 h	8–25	Spherical	Anticancer effect on ovarian cancer cell lines i.e., PA-1, SW-626, and SK-OV-3.	[84]
<i>Dendropanax morbifera</i>	Leaf extract	1 mM chloroauric acid, 80°C for 10 min	10–20	Polygonal and hexagonal	Anticancer activity on HaCaT and A549	[85]
<i>Dracocephalum kotschyi</i>	Leaf extract	1 mM HAuCl_4 , room temperature for 10 min.	11	Spherical	Anticancer activity on K562 and HeLa	[86]
<i>Ecklonia cava</i> (marine brown alga)	Seaweed extract	1 mM chloroauric acid, 80°C , 10 min	20–50	Spherical and triangular	Anticancer activity on HaCaT, MCF-7	[87,88]
<i>Genipa americana</i>	Fruit extract	0.5 mM AuCl_4 solution and kept at $22\text{--}25^\circ\text{C}$, 15 min	30.4 ± 14.9	Spherical	Anticancer activity on A-549 and HeLa	[46,47]
<i>Guazuma ulmifolia</i>	Bark extract	1 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, room temperature, 1 h	20–25	Spherical	Antibacterial properties against <i>Staphylococcus aureus</i> and anticancer activity	[89,90]

Table 1. Cont.

Plant	Used Part	Optimum Synthesis Conditions (Salt Concentration, Temperature, Incubation Time)	Size (nm)	Shape	Applications	Reference
<i>Hibiscus sabdariffa</i>	Leaf extract	(1 mM) H _{Au} Cl ₄ × H ₂ O (100 mL), room temperature, 30 min	10–30	Near spherical	Antifungal potentials against <i>C. krusei</i> , <i>C. guilliermondii</i> , <i>C. glabrata</i> , and <i>C. albicans</i> , antibacterial effects against <i>Streptococcus pneumoniae</i> , <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i> , and <i>Pseudomonas aeruginosa</i> . Anticancer activity on U87 and HEK 293	[91,92]
<i>Justicia glauca</i>	Leaf extract	1 mM chloroauric acid, room temperature, 10 min	32	Hexagonal and spherical	Antimicrobial effects against <i>E. coli</i> , <i>Streptococcus mutans</i> , <i>Micrococcus luteus</i> , <i>S. aureus</i> , <i>S. cerevisiae</i> , <i>Bacillus subtilis</i> , <i>L. acidophilus</i> , <i>P. aeruginosa</i> , and <i>C. albicans</i>	[93,94]
<i>Lantana camara</i>	Fruit extract	0.2 mM AuCl ₄ room temperature (22–25 °C), 72 h	150–300	Triangular	Antibacterial efficacy against <i>S. aureus</i> , <i>E. coli</i> , <i>Propionibacterium acnes</i> , and <i>P. aeruginosa</i>	[48,49]
<i>Linum usitatissimum</i>	Seed extract	1 mM H _{Au} Cl ₄ ·3H ₂ O, room temperature (30 °C), 6 h	3.4–5.7	Spherical and triangular	Anticancer activity on MCF-7, HepG-2, HCT-116	[53]
<i>Lonicera japonica</i>	Flower extract	H _{Au} Cl ₄ concentration (0.125, 0.5, 1, 1.5, and 2 mM), reaction temperature (40, 50, 60, 70, and 80 °C), reaction time (1, 1.5, 2, 2.5, and 3 min)	8	Triangular and tetrahedral	Anticancer activity on HeLa cells	[95]
<i>Mangifera indica</i>	Leaf extract	(5 × 10 ^{−4} M) H _{Au} Cl ₄ ·3H ₂ O, room temperature, 2 min	17–20	Spherical	Anticancer activity on HeLa, MCF-7, Normal fibroblast	[56]
<i>Mangifera indica</i> Linn (Mango)	Peel extract	H _{Au} Cl ₄ (1.0 mM), incubation at 100 °C, 15 min	3.26–21.68	Quasi-spherical	Anticancer activity on CV-1 and WI-38	[57]
<i>Mimosa pudica</i>	Leaf extract	1 mM H _{Au} Cl ₄ ·3H ₂ O, 55 °C, 30 min.	12	Spherical	Anticancer activity on MDA-MB-231, MCF-7 and HMEC	[96]
<i>Musa paradisiaca</i>	Peel extract	1 mM H _{Au} Cl ₄ , 20 min	50	Spherical	Anticancer activity on human lung cancer cells (A549)	[97]
<i>Murraya koenigii</i>	Seed extract	1 mM H _{Au} Cl ₄ , 50 °C, room temperature, 10 min	20–40	Spherical	Antibacterial efficacy against <i>S. aureus</i> , <i>P. aeruginosa</i> and <i>Enterococci</i>	[22,54]
<i>Nerium oleander</i>	Stem/bark extract	1 mM H _{Au} Cl ₄ room temperature (25 °C ± 2 °C), 24 h	20–40	Spherical, hexagonal, triangular, and rod shaped	Anticancer activity on MCF-7 cell lines	[58]
<i>Padina gymnospora</i> (marine Macroalgae)	Leaf extract	1 mM H _{Au} Cl ₄ , 30 °C, 45 °C, 55 °C, 65 °C, 75 °C, 85 °C and 95 °C, few minutes to hours	14.10 ± 1.5	Spherical	Anticancer activity on HepG2, A549, and 3T3 cell line	[98,99]
<i>Platycodon grandiflorum</i>	Leaf extract	H _{Au} Cl ₄ ·3H ₂ O (1 mM), (20, 37, and 50 °C), 10 min	15	Spherical	Antibacterial application against <i>E. coli</i> , <i>B. subtilis</i>	[100]
<i>Phragmites australis</i>	Leaf extract	1 mM H _{Au} Cl ₄ , 85 °C for 1 h	18	Spherical	Anticancer activity on A549 cell line	[101]

Table 1. Cont.

Plant	Used Part	Optimum Synthesis Conditions (Salt Concentration, Temperature, Incubation Time)	Size (nm)	Shape	Applications	Reference
<i>Ricinus ommunis</i>	Leaf extract	HAuCl ₄ (0.5 mM), 60 °C, 5 min	40–80	Spherical	Antibacterial activity against <i>S. aureus</i> , <i>E. coli</i> , <i>P. mirabilis</i> , <i>S. flexneri</i> , <i>C. albicans</i> . Anticancer activity on HT29 and SW480 Cancer Cell	[102]
<i>Pistacia integerrima</i>	Gall extract	1 mM HAuCl ₄ ·3H ₂ O, 37 °C, 24–72 h	20–200	Grain-like	Antibacterial activity against <i>K. pneumonia</i>	[103]
<i>Sargassum swartzii</i>	Seaweed	Chloroauric acid (1 mM HAuCl ₄), 60 °C, 5 min	20–60	Spherical and few hexagonal	Anticancer activity on HeLa	[104]
<i>Terminalia arjuna</i>	Peel extract	1 mM HAuCl ₄ , 80 °C, 15 min	60	Triangular, hexagonal, and pentagonal	Antibacterial activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>S. typhimurium</i>	[105]
<i>Theobromo cacao</i>	Seed extract	1 mM HAuCl ₄ , (30, 40, 50, 60, and 70 °C), 15 min	150–200	Spherical	Antibacterial activity against A431 cell line	[55,106]
<i>Zataria multiflora</i>	Leaf extract	1 mM chloroauric acid (HAuCl ₄), room temperature, few minutes	10–50	Different shapes	Anticancer activity on HeLa and BMSCs cell line	[107]

3. Microbe-Mediated Green Synthesis

In recent years, the prospect of utilizing microorganisms for the environment-friendly synthesis of AuNPs has been introduced [108]. It has been demonstrated that microorganisms act as effective biological agents for the simple, economical, and eco-friendly synthesis of AuNPs, avoiding expensive and hazardous chemicals as well as the high energy requirements of physicochemical methods [109]. There are many recent studies on the green synthesis of AuNPs using microorganisms (Table 2). For green synthesis of AuNPs, a variety of microorganisms are frequently used due to their quick growth, simplicity of handling, and ease of cultivation. These include bacteria, yeast, fungi, and algae [110]. Extracellular and intracellular techniques are two ways to use microorganisms for the environment-friendly synthesis of AuNPs [109]. It is yet unknown exactly how microorganisms help in the production of AuNPs. First, microbial enzymes, such as reductase enzyme, convert the metal ions to nanoparticles (NPs) [111]. Then, a variety of microbial extracellular and intracellular biomolecules act as stabilizing and capping agents [112]. Jafari et al. [113] demonstrated extracellular synthesis of AuNPs from *Micrococcus yunnanensis*. The interaction of 1 mM HAuCl₄ with a bacterial culture supernatant at 30 °C temperature yielded nanoparticles within 24 h of reaction. The size of synthesized AuNPs from FE-TEM analysis was found to range between 15 and 55 nm. Malarkodi et al. [114] also reported the extracellular synthesis of AuNPs using the culture supernatant of a bacterial strain *Klebsiella pneumoniae* within 24 h of reaction at room temperature and found spherical-shaped nanoparticles of 16–50 nm in size.

According to Wen et. al. [115] AuNPs were synthesized through bioreduction of HAuCl₄ by the culture supernatant of *Bacillus megatherium* D01. *Paraclostridium benzolyticum*, *Pseudomonas aeruginosa*, and *Streptomyces viridogens* have the ability to produce AuNPs with greater antimicrobial activity [1,116,117]. Shunmugam et al. [118] has also reported intracellular synthesis of AuNPs using *Vibrio alginolyticus*, but found irregular-shaped nanoparticles of a small size in the range of 50–100 nm. AuNPs were also easily and quickly synthesized in an environmentally friendly way using a variety of fungi and algae. For example, the culture supernatant of *Aspergillus niger* was used to produce AuNPs

with a size of 5.6 ± 12.8 nm [119,120]. The microbial synthesis of AuNPs is also greatly influenced by different parameters, such as salt concentration, extract salt ratio, incubation time, incubation temperature, pH, etc. Cherian et al. [5], investigated the optimum conditions for stable, facile, and rapid synthesis of AuNPs using the culture supernatant of marine bacterium *Lysinibacillus odyseeyi* PBCW2 and they found that various factors such as concentrations of cell-free supernatant and HAuCl_4 , their ratio, pH, incubation temperature, etc., play key roles in the stable and rapid synthesis of AuNPs.

Table 2. Microbe-mediated green synthesis and potential antibacterial and anticancer applications of AuNPs.

Microbes Used for Synthesis	Synthesis Method	Optimum Synthesis Conditions (Salt Concentration, Temperature, Incubation Time)	Size (nm)	Shape	Applications	Reference
<i>Streptomyces</i> sp. ASM19	Extracellular	1 mM HAuCl_4 , 37 °C for 24 h	6.28 ± 0.78 to 100.2 ± 0.25	Sphere-like form	Antimicrobial activity against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> , anticancer potency against liver, colon, breast, and oral carcinoma	[121]
<i>Streptomyces monashensis</i> MSK03	Extracellular	1 mM HAuCl_4 , 37 °C for 72 h	7.1–40.0	Spherical	Antibacterial activity against <i>Pseudomonas aeruginosa</i> and <i>Acinetobacter baumannii</i>	[122]
<i>Alternaria alternate</i>	Extracellular	1 mM HAuCl_4 , room temperature for 24 h	12–29	Spherical, triangular, and hexagonal	Antibacterial application against <i>E. coli</i> and <i>S. aureus</i>	[123]
<i>Aspergillus flavus</i>	Extracellular	10 mM HAuCl_4 , 30 °C for 2 min	12	Spherical	Anticancer agent against HepG2 and A549 cell lines	[124]
<i>Aspergillus clavatus</i>	Extracellular	1 mM HAuCl_4 , room temperature for 48–72 h	24.4 ± 11	Triangular, spherical, and hexagonal	Antibacterial application against <i>E. coli</i> and <i>S. aureus</i>	[125]
<i>Aspergillus foetidus</i>	Extracellular	1 mM HAuCl_4 , 75 ± 2 °C for 4 h	30–50	Spherical	Anticancer effect on A549	[126,127]
<i>Aspergillus niger</i>	Extracellular	1 mM HAuCl_4 , 25 °C for 72 h	5.6 ± 12.8	Spherical	Antibacterial application against <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> . Anti larval application against mosquito larvae	[119,120]
<i>Aspergillus sydowii</i>	Extracellular	3 mM HAuCl_4 , 27 °C for 72 h	8.7–15.6	Spherical	Antibacterial application against <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i>	[128]
<i>Bacillus flexus</i>	Extracellular	1 mM aqueous HAuCl_4 , room temperature for 2 h	20	Different shapes (irregular, spherical, and triangular)	Anticancer effect on MCF-7	[129]
<i>Bacillus megatherium</i>	Extracellular	10 mg/mL HAuCl_4 , 9 h for 26 °C	1.9 ± 0.8	Spherical	Antibacterial application against <i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i>	[115]
<i>Brevibacillus formosus</i>	Extracellular	1 mM HAuCl_4 , 37 °C for 24 h	5–12	Spherical	Antibacterial application against <i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	[130]
<i>Cladosporium</i> sp.	Extracellular	1 mM Chloroauric acid (HAuCl_4), 12 h for 37 °C	5–10	Spherical	Anticancer application against MCF-7	[131]

Table 2. Cont.

Microbes Used for Synthesis	Synthesis Method	Optimum Synthesis Conditions (Salt Concentration, Temperature, Incubation Time)	Size (nm)	Shape	Applications	Reference
<i>Caldicellulosiruptor changbaiensis</i>	Extracellular	500 μ M HAuCl ₄ ·3H ₂ O, 12 h for 75 °C	20–60	Spherical	Antibacterial efficacy against <i>S. aureus</i> and <i>E. coli</i>	[132,133]
<i>Enterococcus</i> sp.	Extracellular	1 mM gold chloride, room temperature for 24–48 h	6–13	Spherical	Anticancer application against HepG2 and A549 cell	[134]
<i>Fusarium solani</i>	Extracellular	1 mM HAuCl ₄ , 28 °C, for 48 h	15–35	Spherical	Anticancer application against Hep2 and Vero cells	[135]
<i>Fusarium oxysporum</i>	Extracellular	0.5 mM HAuCl ₄ , 30 °C for 24 to 96 h	10–40	Spherical	Anticancer application against ZR-75-1, Daudi and PBMC	[136,137]
<i>Humicola</i> spp.	Extracellular	1 mM HAuCl ₄ , 50 °C for 96 h	18–24	Spherical	Anticancer application against NIH3T3 and MDA-MB-231	[138,139]
<i>Micrococcus yunnanensis</i>	Extracellular	1 mM HAuCl ₄ , 30 °C for 24 h	15–55	Spherical	Anticancer application against U87, HT1080, PC12, Caco-2, MCF7, A549. Antibacterial application against <i>B. subtilis</i> , <i>S. typhi</i> , <i>Micrococcus luteus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> ,	[113]
<i>Pseudomonas aeruginosa</i>	Extracellular	1 mM HAuCl ₄ , 37 °C for 24 h	40 \pm 10	Spherical	Antibacterial application against <i>Enterococcus faecalis</i> , <i>S. aureus</i> , and <i>E. coli</i>	[116]
<i>Penicillium brevicompactum</i>	Extracellular	1 mM HAuCl ₄ , 30 °C for 12–72 h	10–120	Spherical, Triangular, and hexagonal	Anticancer application against C ₂ C ₁₂	[140,141]
<i>Pleurotus ostreatus</i>	Extracellular	2.5 mM HAuCl ₄ , 37 °C for 24 h	10–30	Spherical and prism-shaped	Anticancer and synergistic antimicrobial activity against <i>C. albicans</i> , <i>P. aeruginosa</i> and <i>S. aureus</i>	[142,143]
<i>Paracoccus haerundensis</i>	Extracellular	2 mM HAuCl ₄ ·3H ₂ O, 25 °C for 48 h	~20	Spherical	Anticancer application against HaCaT A549	[144]
<i>Rhodopseudomonas capsulata</i>	Extracellular	1 mM aqueous HAuCl ₄ , room temperature for 48 h	10–20	Spherical	Antibacterial application against <i>E. coli</i> and <i>S. aureus</i>	[141,145]
<i>Streptomyces</i> sp.	Extracellular	1 mM aqueous HAuCl ₄ , 80 °C for 30 min	10–50	Spherical and triangular	Anticancer application against HeLa cell	[146]
<i>Streptomyces viridogens</i>	Intracellular	1 mM HAuCl ₄ , 28 °C for 120 h	18–20	Spherical	Antibacterial application against <i>Escherichia coli</i> , and <i>S. aureus</i>	[1,117]
<i>Streptomyces hygroscopicus</i>	Extracellular	1 mM HAuCl ₄ , 30 °C for 48 h	10–20		Antibacterial application against <i>E. coli</i> , <i>S. typhimurium</i> and <i>S. aureus</i> .	[147]
<i>Shewanella oneidensis</i>	Extra cellular	1 mM HAuCl ₄ , 30 °C for 48 h	12 \pm 5	Spherical	Antibacterial application against <i>E. coli</i> and <i>S. aureus</i>	[108,148]
<i>Vibrio alginolyticus</i>	Intracellular	1 mM aqueous chloroauric acid (HAuCl ₄), 40 °C for 24 h	50–100	Irregular	Anticancer application against HCA-7	[118]

4. Characterization of Green Synthesized AuNPs

Various instruments such as ultraviolet-visible spectroscopy (UV-Vis), transmission electron microscopy (TEM), atomic force microscopy (AFM), field emission scanning elec-

tron microscopy (FESEM), X-ray diffraction (XRD), energy dispersive spectroscopy (EDS), Fourier-transformed infrared spectroscopy (FTIR), dynamic light scattering (DLS), and zeta potential analysis are commonly used for the characterization of green synthesized AuNPs. These instruments provide valuable information on the physical and chemical properties of AuNPs, including their size, shape, surface charge, crystallinity, and surface functional groups. TEM, FESEM, and AFM have been instrumental in studying the morphology and size of AuNPs. For instance, TEM has been crucial in observing micellar assemblies formed with triblock copolymers acting as soft templates, leading to tiny AuNPs and resulting in micelle–AuNP hybrid assemblies. AFM, on the other hand, has been utilized to optimize the shape and size of AuNPs by employing block copolymers, demonstrating compact aggregates below 10 nm with a mean height. The control of AuNP size at specific locations has been achieved using oriented phases of block copolymers. Spectroscopic methods, specifically UV-visible spectroscopy, have been employed to measure the absorption spectra of individual AuNPs, revealing surface plasmon resonance and indicating the interaction between delocalized electrons on the nanoparticles' surface and the incident beam. The formation of AuNPs can be followed through the shift of the plasmon in UV-Vis spectroscopy. For example, gold nanoparticles synthesized in the presence of water-soluble micelles in Pluronic exhibited surface plasmon bands at approximately 530–535 nm. Additionally, UV-Vis spectra have been crucial in understanding the inter-particle distances of AuNPs in various polymer systems, providing insights into their growth processes. The crystallinity and purity of synthesized nanoparticles have been examined by XRD analysis. DLS and zeta potential analysis provide information on particle size distribution and surface charge, respectively. Fourier-transformed infrared spectroscopy is an important instrument to investigate the functional groups present on the surfaces of nanoparticles [5,7,149].

The field of green synthesis of AuNPs has seen significant advancements, as demonstrated by several notable studies. Abdulwahed et al. [150] and Kureshi et al. [151] explored the utilization of plant extracts, specifically from apple (*Malus viridis*) and pepper (*Capsicum annuum*) peels, as both reducing and stabilizing agents in the eco-friendly synthesis of AuNPs. These AuNPs were extensively characterized using techniques such as EDS, FTIR, XRD, AFM, FESEM, UV-Vis, and zeta potential analysis. Their research not only delved into the physical characteristics of the synthesized AuNPs but also explored their biological effects, specifically in inhibiting breast cancer cell line MCF-7, with varying results based on the plant sources used [150]. Cherian et al. [5] used UV-visible spectrophotometry, SEM, TEM, EDX, XRD, TGA, DLS, zeta potential analysis, and FTIR to characterize green-synthesized AuNPs using the marine bacterium *Lysinibacillus odyseeyi* PBCW2 and investigate their antioxidant and antibacterial activity. UV-visible spectrophotometry analysis showed a distinct single peak at 520 nm (Figure 2A). TEM analysis revealed the spherical shape of green-synthesized AuNPs (Figure 2B). The SAED pattern showed well-defined diffraction spots in the form of rings, indicating the polycrystalline nature of synthesized AuNPs (Figure 2C). An SEM image depicted small-size nanoparticles with similar shapes and minor-to-negligible aggregations (Figure 2D). XRD analysis confirmed the crystalline structure of synthesized AuNPs with an average size of 29.28 nm (Figure 2E). FTIR analysis revealed various biomolecules present in the bacterial extract and on the surface of synthesized AuNPs responsible for the reducing, capping, and stabilizing of the biosynthesized AuNPs (Figure 2F).

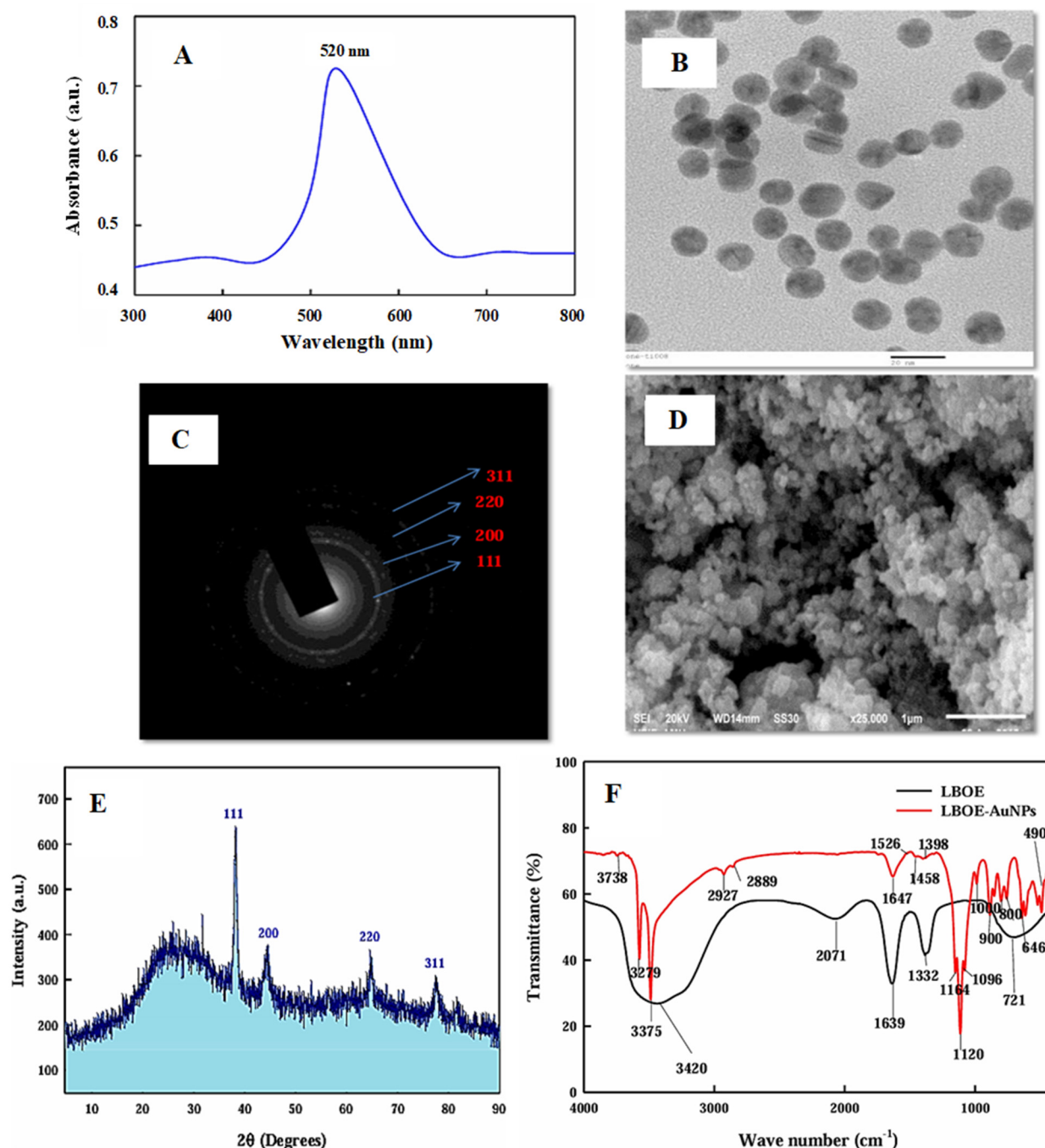


Figure 2. Characterization of LBOE-AuNPs. (A) UV-Vis spectrum, (B) TEM, (C) SAED, (D) SEM, (E) XRD and (F) FTIR spectra of LBOE and LBOE-AuNPs. This figure has been reprinted with permission from ref. [5], copyright 2022, MDPI.

Kureshi et al. [151] utilized aqueous extracts from the fruit pericarps of *Garcinia indica* (GI) and *Garcinia cambogia* (GC) fruits in the green synthesis of AuNPs. This study focused on characterizing the green-synthesized AuNPs, demonstrating their absorption peak at 541 nm through UV-visible spectroscopy. The AuNPs exhibited spherical and triangular shape morphology, with sizes ranging from 2 to 10 nm. These AuNPs demonstrated significant cytotoxicity against the MCF-7 cancer cell line, with IC₅₀ values of 34.55 µg/mL (GI) and 35.69 µg/mL (GC). Moreover, the AuNPs displayed substantial antioxidant and antibacterial properties, showcasing their potential for various biomedical applications [151]. Wang et al. [7] used UV-visible spectrophotometry, FE-TEM, EDX, SAED, XRD, FTIR, TGA, and DLS, to characterize the green-synthesized AuNPs using *Phyllanthus*

emblica fruit extract and *Bifidobacterium animalis* subsp. *lactis* and investigate their anticancer activity against the human gastric carcinoma cell line. UV-visible spectrophotometry analysis showed a distinct single peak at 545 nm, demonstrating the successful synthesis of AuNPs, although there was no peak observed for either the *B. lactis* sample or *Phyllanthus emblica* fruit extract alone, (Figure 3A). Thermogravimetric analysis (TGA) revealed the thermal stability of the green-synthesized AuNPs (Figure 3B). FTIR analysis showed various functional groups present in the *Phyllanthus emblica* fruit extract, bacterial extract, gold salt, and on the surface of synthesized AuNPs (Figure 3C). TEM analysis revealed the circular, triangular, and polygonal nanohybrid shapes of green-synthesized AuNPs with 5–60 nm size (Figure 3D,E). EDX analysis indicated that Au was the main element in the synthesized AuNPs (Figure 3F,G). The SAED pattern showed well-defined diffraction spots, metallic characteristics, and circular structure, indicating the crystalline nature of synthesized AuNPs (Figure 3H). XRD analysis also confirmed the crystalline structure of synthesized AuNPs (Figure 3I). DLS analysis revealed the average hydrodynamic size of synthesized nanoparticles based on number, volume, and intensity (Figure 3J).

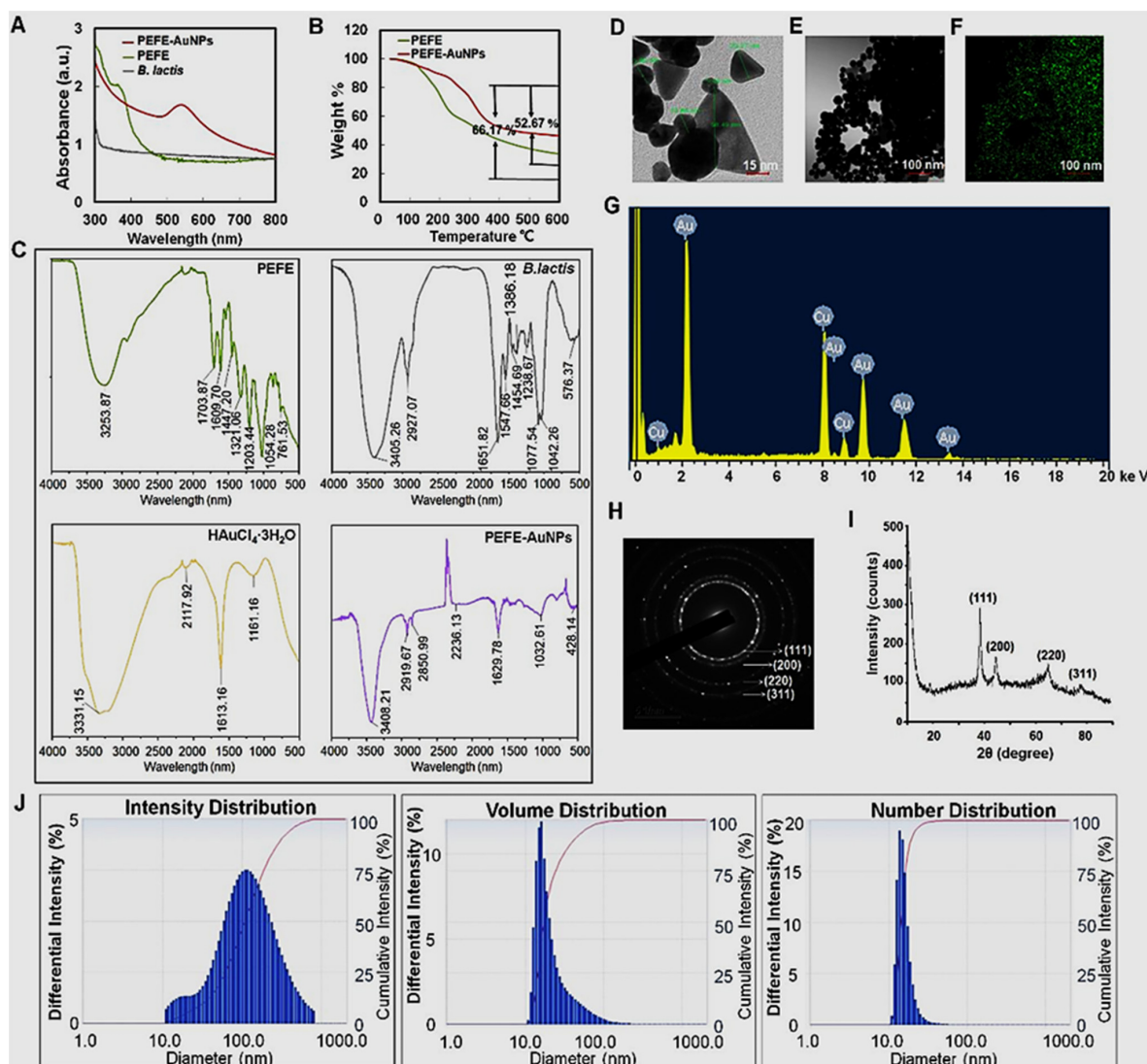


Figure 3. Physiochemical characterization of nanoparticle samples. (A) UV-Vis spectrum, (B) TGA spectrum, (C) FT-IR spectrum, (D) FE-TEM image of PEFE-AuNPs, (E,F) elemental mapping analysis of PEFE-AuNPs, (G) EDX analysis of PEFE-AuNPs, (H) SAED pattern of PEFE-AuNPs, (I) XRD spectrum of PEFE-AuNPs, and (J) DLS spectrum of PEFE-AuNPs. This figure has been reprinted with permission from ref. [7], copyright 2021, MDPI.

5. Antibacterial Applications and Mechanisms of Green-Synthesized AuNPs

The growing menace of antibacterial resistance has underscored the urgency for innovative strategies to combat infectious diseases. Green-synthesized AuNPs have emerged as promising candidates due to their potent antimicrobial activity. AuNPs have been extensively studied for their ability to inhibit the growth of pathogenic microorganisms, including drug-resistant pathogenic bacteria [33,119,123]. Zheng [152] conducted a study highlighting the efficacy of ultrasmall gold nanoclusters (AuNCs) with dimensions less than 2 nm in manifesting a broad-spectrum antimicrobial effect against both Gram-positive and Gram-negative bacteria. The diminutive size of these AuNCs facilitated enhanced interactions with bacterial cells, resulting in metabolic perturbations and heightened generation of reactive oxygen species, thereby inducing bacterial cell death [152]. Contrastingly, Zhang et al. [153] proposed an alternative perspective, suggesting that the antimicrobial activity of AuNPs could be ascribed to co-existing chemical factors, including gold ions and surface coating agents, rather than the intrinsic properties of the nanoparticles themselves. This nuanced interpretation underscores the importance of considering multiple facets when elucidating the antibacterial mechanisms of AuNPs. Moreover, investigations by Lokina et al. [154] delved into the synthesis of AuNPs utilizing *Punica Granatum* fruit extract. The resulting AuNPs exhibited noteworthy antibacterial efficacy against a spectrum of microorganisms, providing a novel and natural source for the fabrication of antimicrobial nanomaterials [154]. Additionally, the multifunctionality of AuNPs was demonstrated by their capacity to augment the bactericidal effects of antibiotics when employed as carriers or delivery vehicles. This finding, as suggested by several studies, opens avenues for synergistic approaches in antimicrobial therapy, wherein AuNPs act as potent adjuncts to conventional antibiotics, enhancing their efficacy [152,154]. The amalgamation of findings from these studies elucidates the diverse mechanisms and applications of AuNPs in the realm of antimicrobial activity, encompassing both size-dependent interactions with bacterial cells and the potential contributions of co-existing chemical constituents. The synthesis methodologies, such as the implementation of natural extracts, further broaden the scope of utilizing AuNPs for combating microbial infections. Cherian et al. [5] reported the green synthesis of AuNPs using the culture supernatant of marine bacterium *Lysinibacillus odyseeyi* PBCW2 and evaluated their antimicrobial activity against several pathogenic bacteria such as *Vibrio cholera* MTCC 3905, *Escherichia coli* serotype 0115, *Aeromonas hydrophila* IDH1585, and *Staphylococcus aureus*. They found that *Lysinibacillus odyseeyi* PBCW2-mediated synthesized AuNPs strongly suppressed the growth of these pathogenic bacteria and showed a strong zone of inhibition (Figure 4).

Zhao [155] contributes to the elucidation of the antibacterial mechanisms of gold nanoparticles (AuNPs) by demonstrating that pyrimidine-capped gold nanoparticles can disrupt bacterial cell membranes. This disruption leads to the leakage of cytoplasmic contents and subsequent inhibition of protein synthesis. The study sheds light on the specific molecular interactions between AuNPs and bacterial cells, underscoring the importance of membrane disruption as a key aspect of AuNPs' antibacterial activity [155]. In a different vein, Lee and Lee [156] emphasized the synergistic antibacterial activity of AuNPs, although the explicit mechanism underlying this synergy is not explicitly discussed. The study hints at complex interactions between AuNPs and bacterial cells, suggesting that the antibacterial effects may result from a combination of multiple factors. The exploration of this synergistic antibacterial activity opens avenues for further investigation into the nuanced interplay between AuNPs and bacterial pathogens [156]. Timoszyk and Grochowalska [60] delved into the antibacterial activity of AuNPs functionalized with natural compounds derived from plants. The study underscores the significance of natural

coating in influencing interactions with bacterial cell walls, providing insights into the role of surface modifications in enhancing antibacterial efficacy. This approach highlights the potential of using plant-derived compounds to functionalize AuNPs for optimized antibacterial applications [60]. Mahdi and Parveen [157] focused on the biosynthesis of AuNPs using black lemon extract and investigated their antibacterial activity against both Gram-positive and Gram-negative bacteria. The study showcases a bioinspired approach to AuNP synthesis and emphasizes the broad-spectrum antibacterial efficacy of the resulting nanoparticles. This work contributes to the expanding repertoire of environmentally friendly methods for AuNP synthesis with potent antibacterial applications [157].

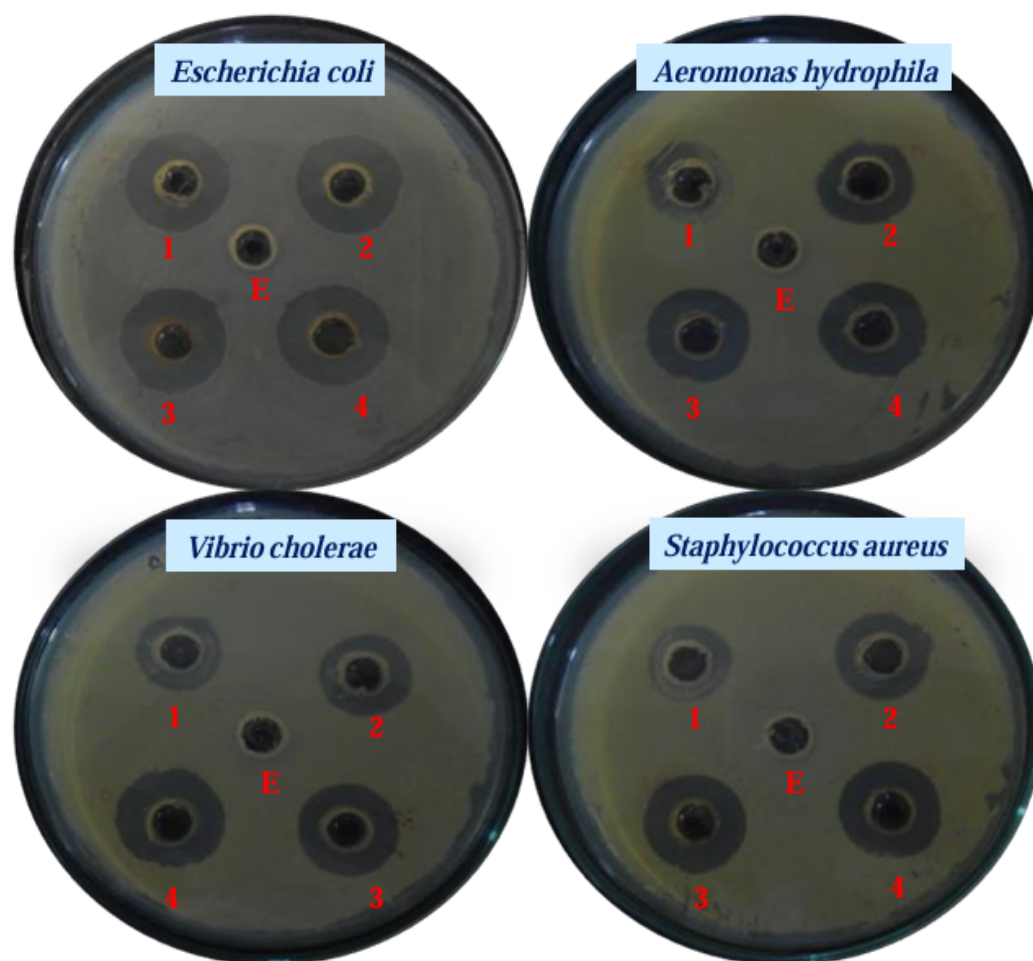


Figure 4. Antibacterial activity of LBOE (E) and LBOE-AuNPs at (1) 20, (2) 60, (3) 80, and (4) 100 µg/mL based on the agar well diffusion assay. This figure has been reprinted with permission from ref. [5], copyright 2022, MDPI.

Dasari et al. [158] explored the antibacterial activity of Au(I) and Au(III) against different bacteria, revealing their toxicity to both nonpathogenic and multidrug-resistant strains. Buffer composition significantly affected bacterial growth inhibition, highlighting the importance of experimental conditions when studying AuNP toxicity. Centrifugation-resuspension successfully removed residual Au(III) ions from AuNPs, making them non-toxic. Wang et al. [159] developed a photosensitizer-loaded hybrid nanostructure with high antibacterial efficiency. AuNPs on the polymer surface amplified the photosensitizer's effects, leading to increased reactive oxygen species (ROS) generation. The nanostructure exhibited significant antibacterial efficacy against *Escherichia coli* in the presence of lectin protein, providing an innovative approach for controlled antibacterial assays. Khan et al. [160] employed biogenic synthesis using *Acer pentapomicum* leaf extract to produce AuNPs. These

nanoparticles exhibited spherical morphology and demonstrated antibacterial, antifungal, and antioxidant activities, showcasing their potential in various applications. Mahmoud et al. [161] developed a library of gold nanorods (GNRs) with different coatings, investigating their penetration into skin layers. Cholesterol-PEG-coated GNRs showed preferential accumulation in the upper skin layers, demonstrating antibacterial activity against *Staphylococcus aureus*. These findings open new avenues for utilizing gold-based nanoscale systems in skin disease therapy [161]. These studies collectively emphasize the diverse strategies and conditions impacting the antibacterial properties of AuNPs, showcasing their potential in various biomedical applications.

In summary, these studies collectively underscore the multifaceted nature of AuNPs regarding antibacterial activity. The mechanisms include membrane disruption, inhibition of protein synthesis, interactions with bacterial cell walls, and potentially synergistic effects. This diverse range of antibacterial mechanisms highlights the versatility of AuNPs and their potential in developing effective strategies against bacterial infections. One of the key mechanisms underlying the antibacterial action of AuNPs involves their ability to disrupt the bacterial cell membrane integrity. These nanoparticles can easily penetrate the bacterial cell wall, leading to membrane damage and subsequent cell death. The small size and large surface area of AuNPs enhance their interactions with bacterial cells, allowing for efficient binding and disruption of the cell membrane. Additionally, AuNPs can generate reactive oxygen species (ROS) upon interaction with bacterial cells. ROS, such as superoxide radicals and hydrogen peroxide, cause oxidative stress and damage to cellular components, leading to bacterial cell death. Moreover, AuNPs can interfere with bacterial enzymes and proteins, disrupting essential cellular processes. Another intriguing aspect of the antibacterial mechanism of AuNPs is their ability to inhibit biofilm formation. Biofilms, which are communities of bacteria encased in a protective extracellular matrix, are notoriously resistant to conventional antibiotics. AuNPs have shown the potential to prevent biofilm formation and disrupt pre-formed biofilms, making them valuable candidates for combating persistent bacterial infections. Furthermore, AuNPs can be functionalized with various antibacterial agents, enhancing their efficacy and specificity against specific bacterial strains. The surface modification of AuNPs with molecules like antibiotics, peptides, or polymers allows for targeted delivery of antibacterial agents to specific bacterial cells while minimizing damage to healthy cells, thus reducing side effects. The versatility of AuNPs in terms of size, shape, and surface chemistry further contributes to their wide-ranging antibacterial applications. Researchers continue to explore innovative methods to optimize AuNPs' antibacterial properties and develop novel strategies for combating antibiotic-resistant bacteria. As the understanding of AuNPs' mechanisms of action deepens, their potential to revolutionize antibacterial therapy becomes increasingly evident, offering hope for more effective and targeted treatments against infectious diseases. Figure 5 shows the proposed antibacterial mechanisms of AuNPs.

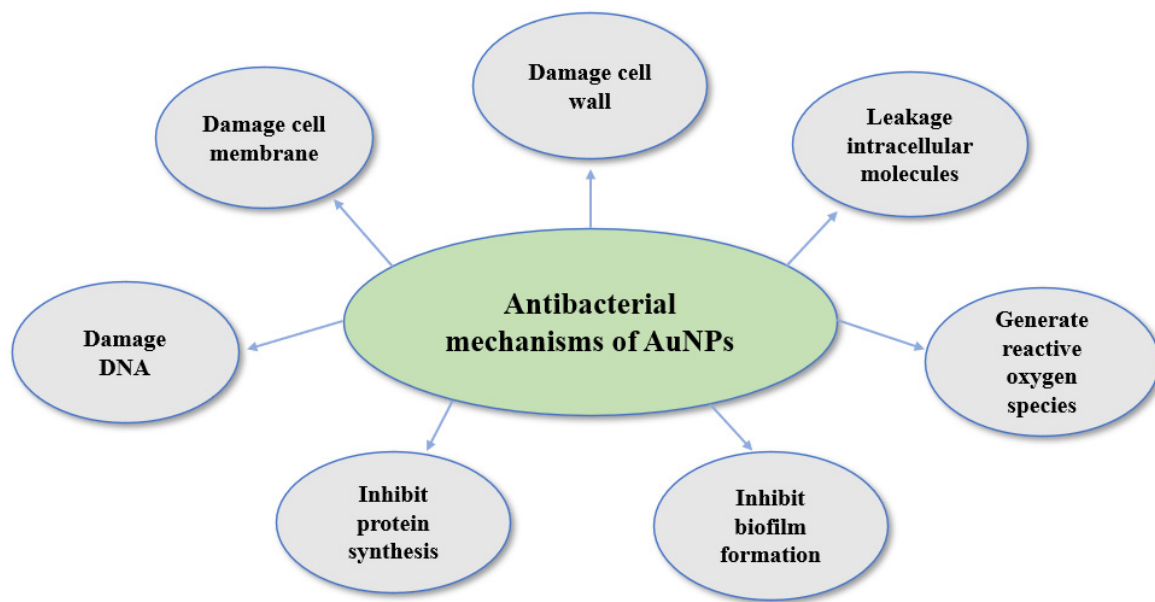


Figure 5. Proposed antibacterial mechanisms of AuNPs.

6. Anticancer Applications and Mechanisms of Green Synthesized AuNPs

The fight against cancer is a major global challenge, driving relentless efforts to discover safe and effective anticancer drugs. Green-synthesized AuNPs could be a potential game-changer in this case. Many studies have reported the strong anticancer efficacy of green-synthesized AuNPs against different cancer cell lines [22,34,42,126,162]. Barai and colleagues reported that AuNPs synthesized from *Nerium oleander* bark extract induced an anti-breast cancer effect in MCF-7 cell lines [58]. Also, grapefruit extract-derived AuNPs showed anticancer properties in HeLa cell lines. Eight green botanical extracts of different plant components were used to create AuNPs, and their anticancer properties were examined against MCF 7 (breast cancer) cell lines [163]. *Cirsium japonicum* extract was used in the biological synthesis of AuNPs, which displayed anticancer impacts on stomach cancer (AGS cell lines) [164]. According to research, AuNPs from plant extracts of *Catharanthus roseus* and *Carica papaya* may be able to treat acute lymphocytic leukemia, breast cancer, Hodgkin's disease, leukemia, lymphomas, neuroblastoma, soft tissue sarcomas, and multiple myeloma [165]. Similarly, *Citrus macroptera* fruit extract was used to produce AuNPs, which were then tested for anticancer action on three distinct malignant cell lines: MDA-MB 468 (breast cancer cell), tA 549 (alveolar basal epithelial cells), and HepG2 (liver cancer cell line) [166]. Furthermore, extracts of *Taxus baccata*, *Marsdenia tenacissima*, *Lonicera japonica*, *Abies spectabilis*, *Gymnema sylvestre*, *Panax notoginseng*, and *Sasa borealis* were used for the green synthesis of AuNPs and the synthesized AuNPs exhibited potential anticancer effects in the following cancerous cell types: the HT29 cell line [167], PANIC-1 cells [168], AGS cell lines [169], lung cancer A549 cells [170], cervix cancer (HeLa) cells [95], bladder cancer T24 cell lines [171], cervical cancer HeLa cells [172], breast adenocarcinoma (MCF-7) cancer cells [173], and various cancerous cells like breast (MCF-7), cervical (HeLa), and ovarian (Caov-4) [174]. In a 2023 study, G Tan et al. reported garlic and onion extract-mediated green synthesis of AuNPs and their anticancer activity in MCF-7 cells [175]. In addition, *Halymenia pseudofloresii* extract, bovine serum albumin, *Hypnea valentiae* seaweed, *Sonneratia alba* fruits, jellyfish nematocyst venom protein, and *Nothapodytes foetida* leaf extract were used for the green synthesis of AuNPs and displayed potential anticancer effects in lung cancer cells [176], cervix cancer cells (HeLa) [177], lung cancer A549 cells [178], lung cancer

cell line A549 [179], breast cancer MCF-7 cells [180], cervix cancer cell line KB-3-1 [181], and lung cancer A549 cell lines [182].

The antitumor effect of AuNPs against liver and cancer cells was investigated using marine microbe *Enterococcus* sp.-mediated green-synthesized AuNPs [134]. AuNPs synthesized by the bacteria *Streptomyces griseus* displayed anti-breast (MCF-7 cell lines) and colon (HCT-116 cell lines) cancer impacts [183]. *Exiguobacterium aestuarii*-mediated green-synthesized AuNPs showed apoptosis activity in human breast cancer MCF-7 cells [184]. AuNPs synthesized with red algae (*Halymenia pseudofloresii*) trigger apoptosis in lung cancer cells by ROS formation [176]. *Commiphora wightii* plant conjugated with AuNPs and fungus (*Cladosporium* sp.) showed an anticancer effect against breast (MCF-7 cell lines) cancer [131]. In MCF10A and MCF7 cancer cells, AuNPs were conjugated with *Microalga dunaliella* (which is halotolerant algae) to show anticancer action using green technology [185]. AuNPs were synthesized by *Sargassum glaucescens* (brown seaweed), and their activity checked in the breast (MDA-MB-231), cervical (HeLa), leukemia (CEM-ss), and liver (HepG2) cancerous cell lines [186]. In another report, extract of *Sargassum incisifolium* was employed to make AuNPs and confirm their toxicity in cancer cells (MCF-7 and HT-29), which does not appear to be harmful to the environment [187]. Wang et al. [7] reported the green synthesis of AuNPs using both the fruit extract of *Phyllanthus emblica* and culture supernatant of *Bifidobacterium animalis* subsp. *lactis* and investigated their anticancer activity against human gastric carcinoma cell lines. They found that *Phyllanthus emblica* fruit extract and *Bifidobacterium animalis* subsp. *Lactis*-mediated green-synthesized AuNPs strongly suppressed the growth of the human gastric adenocarcinoma cell line (AGS). They concluded that the anticancer activity of synthesized AuNPs against gastric cancer cells was associated with the induction of apoptosis through inhibition of autophagy, downregulation of LC3-II/LC3-I and Beclin-1 expression, and upregulation of p62 expression in AGS cells [7]. Figure 6 shows the green synthesis of gold nanoparticles using *Phyllanthus emblica* fruit extract and *Bifidobacterium animalis* subsp. *lactis* and their anticancer effect on human gastric cancer cells.

The broad surface area of AuNPs makes them highly capable of mixing, enabling them to be conjugated to various compounds, including anticancer agents and other biomolecules [163]. Since most capillaries have pores that range in size from 6 to 12 nm, the size of the nanoparticles makes them simpler for tumor cell membranes to penetrate. Therefore, bigger nanoparticles will facilitate simpler extravasation. Additionally, tumor arteries typically include pores that range in size from 40 to 200 nm [188]. Therefore, the efficacy of nanoparticles can be increased by conjugating these nanoparticles with other anticancer agents. For instance, anti-VEGF protein conjugated with AuNPs is being revealed to enhance the apoptosis and cell development in CLL cells in comparison to their antibody [189]. AuNPs achieve maximum efficacy, minimal side effects, and reduce damage to normal (noncancerous) cells [164]. AuNPs show synergistic cancer therapeutic [190] and cytotoxic effects against cancerous cells depending on the nanoparticle dose [191]. Conjugation of bioactive AuNPs with natural ligands could be a good idea for the development of novel, safe, and effective anticancer agents. Several studies have reported on the conjugation of bioactive AuNPs with various ligands including polyethylene glycol, folic acid, hyaluronic acid, naturally occurring proteins, and peptides, etc. [192,193]. Conjugation of these ligands with bioactive AuNPs aids in targeted delivery, minimizes cytotoxic effects on normal cells and increases anticancer efficacies [192,193].

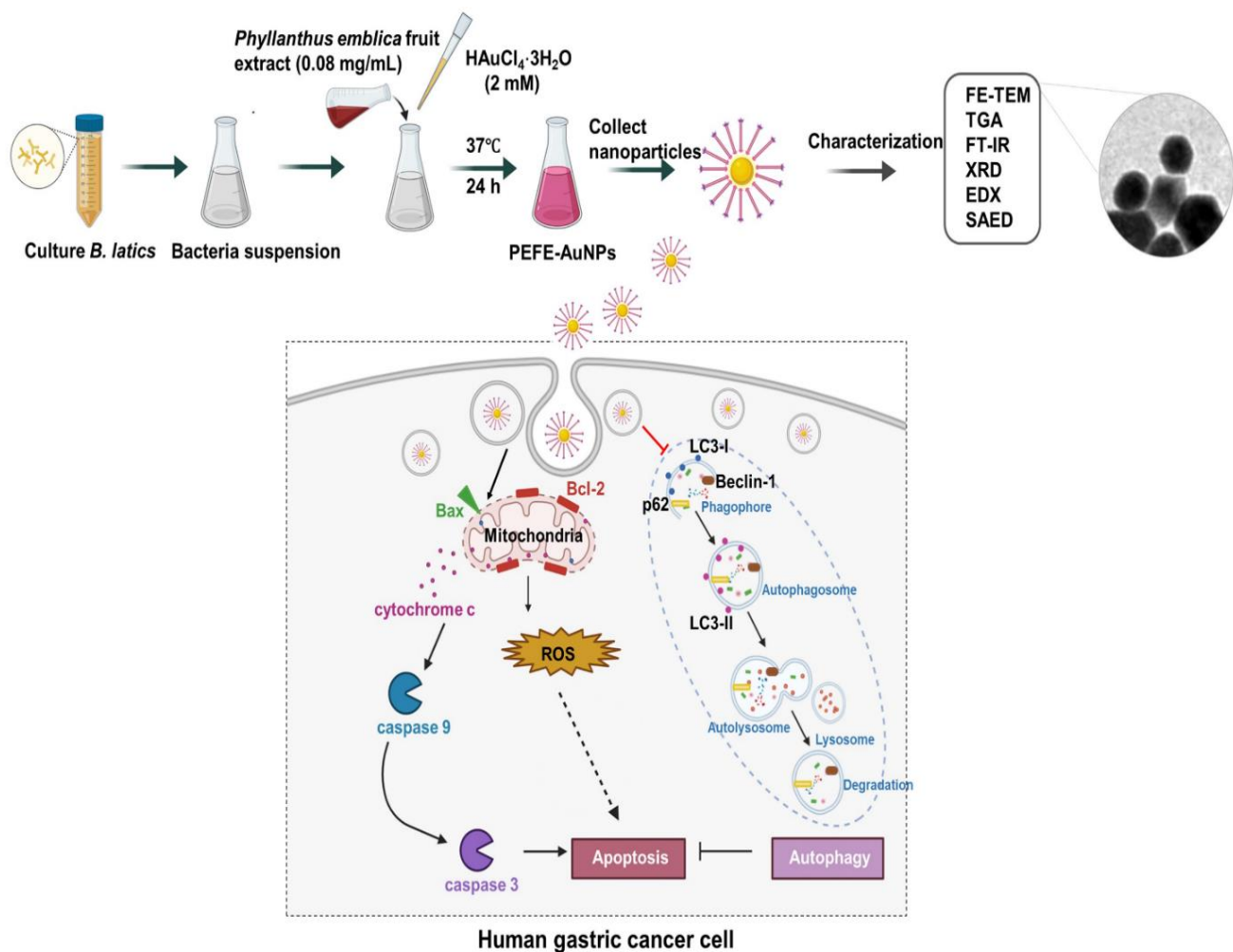


Figure 6. Green synthesis of gold nanoparticles using *Phyllanthus emblica* fruit extract and *Bifidobacterium animalis* subsp. *lactis* and their anticancer effect on human gastric cancer cells. This figure has been reprinted with permission from ref. [7], copyright 2022, MDPI.

The anticancer effect of AuNPs is attributed to a complex and poorly understood mechanism. Several molecular mechanisms of anticancer activity of green-synthesized AuNPs have been reported. These mechanisms include induction of apoptosis, mitochondrial dysfunction, inhibition of angiogenesis, and generation of reactive oxygen species, etc. [194]. AuNPs are used for component transporters and exhibit an anticancer effect [191]. Researchers have reported that the interaction of AuNPs with cells occurs in different ways, such as cellular internalization of AuNPs [195]. AuNP properties of the surface are significant factors in cell uptake. Cancer and normal cell membranes contain negatively charged substances such as lipids (particularly phosphate), and AuNPs with positive charges are taken up and internalized by these membranes with opposite charges [196,197]. Endocytosis is another method for AuNPs to enter cells, as demonstrated by a study in which small AuNPs were endocytosed and aggregation was observed in HeLa cells [198]. The AuNPs exhibited cytotoxic action through ROS formation [199], damage to DNA [191], and triggering of caspase cascade, resulting in apoptotic and mitochondrial abnormalities [164,199]. According to reports, ROS generation, mitochondrial malfunction, and apoptosis triggered by caspase are caused by AuNPs mediated by *phyllanthus emblica* extract [7].

Rhus chinensis plant extract-conjugated AuNPs have shown effects on multiple cancerous cells, exhibiting cytotoxic action through DNA damage [200]. *P. hexandrum* AuNPs

produced comparable effects on HeLa cells [191]. Apoptosis induced by activation of caspase cascades, which includes caspase cascades 3, 8, and 9, and cell cycle arrest in the G2/M phase was also seen [201]. *Moringa oleifera*-conjugated AuNPs in lung cancer A549 cells were also observed to exhibit caspase-mediated apoptosis, as evidenced by an increase in caspase 3/7 and 9 activity, a substantial decrease in ATP levels, and a noticeable rise in Bax and p53 levels of proteins [202]. Figure 7 shows the proposed mechanisms of action of green-synthesized AuNPs against cancer cells.

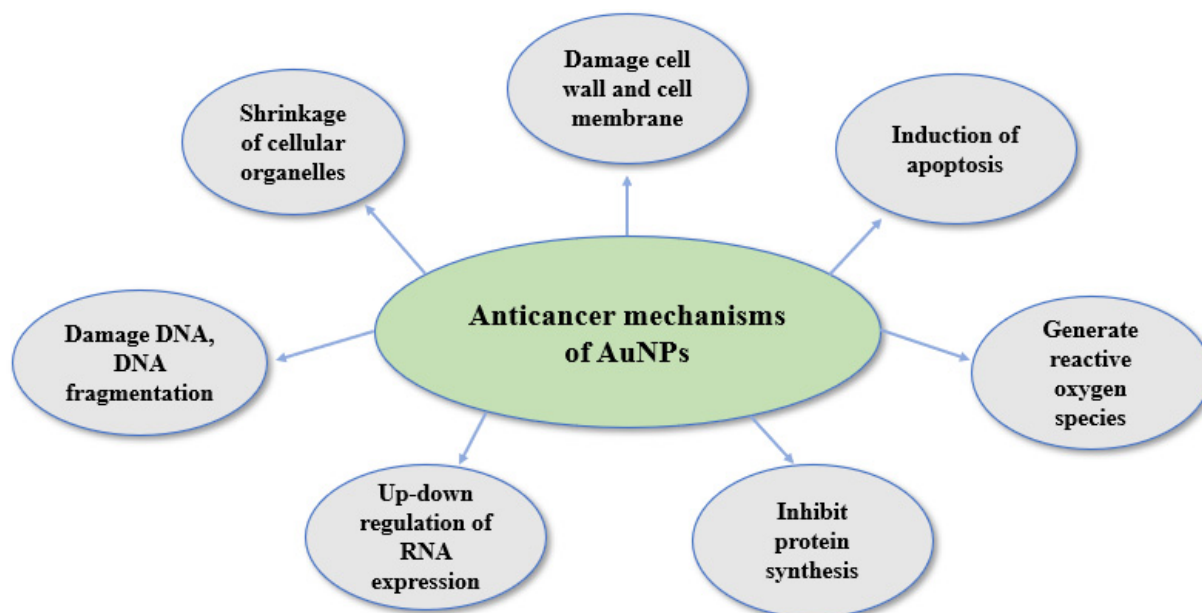


Figure 7. Proposed mechanisms of action of green-synthesized AuNPs against cancer cells.

7. Conclusions and Future Prospects

The emergence of antibacterial and anticancer drug resistance severely threatens global public health. The growing menace of antibacterial and anticancer drug resistance has underscored the urgency for innovative strategies to combat cancer and infectious diseases caused by pathogenic microorganisms. Hence, it is essential to develop novel, safe, and effective antimicrobial and anticancer agents. Bioactive AuNPs could be promising agents to solve this problem. Recently, many studies have been conducted on the green synthesis of AuNPs using either plants or microorganisms and investigated their potential application against drug-resistant pathogenic bacteria as well as cancer cells. The present review provides an overview of the green synthesis of AuNPs using plants and microorganisms and their potential applications against drug-resistant pathogenic bacteria and cancer cells. Moreover, this review focuses on the antibacterial and anticancer mechanisms of facile and eco-friendly synthesized AuNPs. Green synthesis of AuNPs using plants and microbes is a facile, non-toxic, cost-effective, and eco-friendly method. Plant extracts contain various bioactive compounds, and microbial extracts also contain numerous metabolites that act as reducing, capping, and stabilizing agents during the synthesis of AuNPs. The antibacterial activity of green-synthesized AuNPs is attributed to several mechanisms, including morphological and structural changes, damage to the cell wall and cell membrane, leakage of intracellular molecules, damage to DNA, inhibition of protein synthesis, inhibition of biofilm formation, generation of reactive oxygen species, etc. Similarly, the anticancer activity of green-synthesized AuNPs is attributed to several mechanisms, including damage to the cell wall and cell membrane, shrinkage of cellular organelles, damage to DNA, up-down regulation of RNA expression, inhibition of protein synthesis, induction of apoptosis, generation of reactive oxygen species, etc.

Although many reports have already been published on the green synthesis of AuNPs for the development of antimicrobial and anticancer agents, some challenges remain. The challenges include large-scale production, long-term stability, biocompatibility, targeted delivery to minimize cytotoxic effects on normal cells, and the absence of clear clinical trial data. Several points might be considered for the future synthesis of AuNPs to address these challenges. First of all, the selection of a plant or microbial extract that contains antibacterial or anticancer-active biomolecules. If the plant or microbial extract contains antibacterial or anticancer-active biomolecules, the efficacy of green-synthesized AuNPs could be greatly increased. Second, the optimization of various synthesis parameters, such as salt concentration, plant or microbial extract concentration, extract salt ratio, incubation time, incubation temperature, pH, etc., is very important for high, stable, and rapid synthesis of AuNPs. Third, the conjugation of green-synthesized antibacterial or anticancer-active AuNPs with antibacterial or anticancer drugs or other antibacterial or anticancer-active biomolecules could be a great approach for the development of novel and effective antibacterial and anticancer drugs. Conjugation of bioactive AuNPs with ligands could be a good idea for targeted delivery to minimize cytotoxic effects on normal cells. Fourth, the potential adverse effects of green-synthesized antibacterial and anticancer-active AuNPs on human health and the environment should be studied. It is very important to investigate the biocompatibility and biosafety of synthesized AuNPs using a proper in vitro and in vivo screening model system. Finally, clinical trials should be conducted for the development of novel, safe, and effective antibacterial or anticancer-active AuNPs.

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